United States Department of the Interior
Bureau of Land Management

Supplemental Information
For the
Little Fish Lake Joint Management Area
Wild Horse Gather Plan
Environmental Assessment
DOI-BLM-NV-B020-2022-0030-EA

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U.S. Department of the Interior
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1.0 Regulatory Framework

Wild and Free-Roaming Horse and Burro Act of 1971

The Wild Free-Roaming Horses and Burros Act of 1971 (WFRHBA) (Public Law 92-195) protects wild free-roaming horses and burros from capture, branding, harassment, or death. Wild horses are to be managed to achieve and maintain a thriving natural ecological balance on public lands. Additionally, management of wild horses and burros is to be undertaken to protect the range from deterioration associated with overpopulation of wild and free-roaming horses.

Tonopah ROD and RMP

The Tonopah RMP and ROD (BLM, 1997) objectives for wild horses and burros include:

"To manage wild horse and/or burro populations within Herd Management Areas at levels which will preserve and maintain a thriving natural ecological balance consistent with other multiple-use objectives."

Long-term management action for wild horses and burros in the RMP and ROD (BLM, 1997) include:

- Manage wild horses and burros in 16 HMAs.
- Manage wild horse and/or burros at appropriate management level (AML) or interim herd size for each HMA. Future herd or appropriate management levels within each HMA will be adjusted as determined through short-term and long-term monitoring data methods.
- Assure sufficient water and forage exist for wild horses and/or burros in HMAs.
- When the appropriate management level (or in some cases interim herd size) is exceeded, remove excess wild horses and/or burros to a point which may allow up to three years of population increase before again reaching the appropriate management level or interim herd size.
- Apply for appropriate water rights and/or assert public water reserves on water sources as necessary to ensure a reliable, year-round water source for wild horses and burros in HMAs.

Approved RMP Amendments

In 2015, the BLM released a ROD and Approved RMP Amendments (ARMPA) for the Great Basin Region, including the Greater Sage-Grouse (GRSG) Sub-Regions of Idaho and Southwestern Montana, Nevada and Northeastern California, Oregon, and Utah.

Management Decisions (MD):

MD Wild Horse and Burros (WHB) 1: For WHB management activities (e.g., gathers), review Objective Special Status Species (SSS) 4 and apply MDs SSS 1 through SSS 4 when reviewing and analyzing projects and activities proposed in GRSG habitat.

MD WHB 2: Manage HMAs in GRSG habitat within established AML ranges to achieve and maintain GRSG habitat objectives.

MD WHB 3: Complete rangeland health assessments for HMAs containing GRSG habitat using an interdisciplinary team of specialists (e.g., range, wildlife, and riparian). The priorities for conducting assessments are:

- HMAs containing Priority Habitat Management Areas (PHMAs), which include riparian areas.
- HMAs containing only General Habitat Management Area (GHMAs).
- HMAs containing sagebrush habitat outside of PHMA and GHMA mapped habitat.

- HMAs without GRSG habitat.

MD WHB 4: Prioritize gather and population growth suppression techniques in HMAs in GRSG habitat, unless removals are necessary in other areas to address higher priority environmental issues, including herd health impacts. Place higher priority on HAs not allocated as HMAs and occupied by wild horses and burros PHMAs.

MD WHB 5: In PHMAs, assess and adjust AMLs through the National Environmental Policy Act (NEPA) process within HMAs when wild horses or burros are identified as a significant causal factor in not meeting rangeland health standards, even if current AML is not being exceeded.

MD WHB 6: In PHMAs, monitor the effects of WHB use in relation to GRSG habitat objectives on an annual basis to help determine future management actions.

MD WHB 7: Develop or amend HMA plans to incorporate GRSG habitat objectives and management considerations for all HMAs within GRSG habitat, with emphasis placed on PHMAs.

MD WHB 8: Consider removals or exclusion of WHB during or immediately following emergency situations (such as fire, floods, and drought) to facilitate meeting GRSG habitat objectives where HMAs overlap with GRSG habitat.

MD WHB 9: When conducting NEPA analysis for wild horse/burro management activities, water developments, or other rangeland improvements for wild horses, address the direct and indirect effects to GRSG populations and habitat. Implement any water developments or rangeland improvements using the criteria identified for domestic livestock.

MD WHB 10: Coordinate with professionals from other federal and state agencies, researchers at universities, and others to utilize and evaluate new management tools (e.g., population growth suppression, inventory techniques, and telemetry) for implementing the WHB program.

Mojave/Southern Great Basin Resource Advisory Council Standards and Guidelines

From the preamble to the Standards and Guidelines for Wild Horse and Burro Management:

"The standards for rangeland health will be reached and maintained by managing wild horse and burro numbers so as not to exceed Appropriate Management Levels (AML) for each HMA. Controlling wild horse and burro numbers through gathers and other control programs is essential."

Guidelines for the Wild Horses and Burros Standard include:

4.1 Wild horse and burro population levels in HMAs should not exceed AML.
…
4.7 Wild horse and burro herd management practices should address improvement beyond this standard, significant progress toward achieving standards, time necessary for recovery, and time necessary for predicting trends.

2.0 Pertinent Wild Horse & Burro Program Regulations

From Title 43, Part 4700 of the Code of Federal Regulations (CFR):

§4720.1 Removal of excess animals from public lands.

Upon examination of current information and a determination by the authorized officer that an excess of wild horses or burros exists, the authorized officer shall remove the excess animals immediately in the following order.

(a) Old, sick, or lame animals shall be destroyed in accordance with subpart 4730 of this title;

(b) Additional excess animals for which an adoption demand by qualified individuals exists shall be humanely captured and made available for private maintenance in accordance with subpart 4750 of this title; and

(c) Remaining excess animals for which no adoption demand by qualified individuals exists shall be destroyed in accordance with subpart 4730 of this title.

§4730.1 Destruction.

Except as an act of mercy, no wild horse or burro shall be destroyed without the authorization of the authorized officer. Old, sick, or lame animals shall be destroyed in the most humane manner possible. Excess animals for which adoption demand does not exist shall be destroyed in the most humane and cost-efficient manner possible.

§4740.1 Use of motor vehicles or aircraft.

(a) Motor vehicles and aircraft may be used by the authorized officer in all phases of the administration of the Act, except that no motor vehicle or aircraft, other than helicopters, shall be used for the purpose of herding or chasing wild horses or burros for capture or destruction. All such use shall be conducted in a humane manner.

(b) Before using helicopters or motor vehicles in the management of wild horses or burros, the authorized officer shall conduct a public hearing in the area where such use is to be made.

§4740.2 Standards for vehicles used for transport of wild horses and burros.

(a) Use of motor vehicles for transport of wild horses or burros shall be in accordance with appropriate local, State and Federal laws and regulations applicable to the humane transportation of horses and burros, and shall include, but not be limited to, the following standards:

(1) The interior of enclosures shall be free from protrusion that could injure animals;

(2) Equipment shall be in safe conditions and of sufficient strength to withstand the rigors of transportation;

(3) Enclosures shall have ample head room to allow animals to stand normally;

(4) Enclosures for transporting two or more animals shall have partitions to separate them by age and sex as deemed necessary by the authorized officer;

(5) Floors of enclosures shall be covered with nonskid material;

(6) Enclosures shall be adequately ventilated and offer sufficient protection to animals from inclement weather and temperature extremes; and

(7) Unless otherwise approved by the authorized officer, transportation shall be limited in sequence to a maximum of 24 hours followed by a minimum of 5 hours of on-the-ground rest with adequate feed and water.

(b) The authorized officer shall not load wild horses or burros if he/she determines that the vehicle to be used for transporting the wild horses or burros is not satisfactory for that purpose.

3.0 WinEquus Population Modeling

Overview

The WinEquus Feral Horse Population Model, developed by Dr. Steven Jenkins at the University of Nevada at Reno, was designed to assist Wild Horse and Burro Specialists evaluate various management plans and possible outcomes for management of wild horses that might be considered for a particular area.

The purpose of the modeling was to compare the potential results of the Proposed Action and Alternatives including the No Action to include population size over time, growth rates, and the number of animals that could be gathered, removed and treated for fertility control over the next 10 years.

The model was run for 10 years to show potential effects over time. However, prior to future gathers, the data from this proposed gather along with future inventory data would be analyzed to determine the appropriate course of action. Appropriate NEPA would also be completed, if necessary, prior to a future gather being conducted.

The current WinEquus Population Model includes options for management by Fertility Control Only, Removals only or Removals and Fertility Control. The model was created to show implementation of all of the management through actual gathers, removals and treatment of horses. Currently, within WinEquus, there are no options to implement booster treatment of fertility control via darting, initial or repeat treatment of PZP-22 via bait and water trapping, or gelding. Because of these limitations, the results for the modeling provide a general idea of the range of potential outcomes.

Because of the way the population model reflects the first foaling season at the beginning of the trial, the initial gather year was set to 2021 to reflect first foaling in 2022.

The Proposed Action involves the use of fertility control. Alternative 1 was shown to manage through removals only, with no fertility control. The No Action alternative includes no management, removals or fertility control to simulate continued growth of the population. The fertility control only alternative includes management solely through the use of fertility control; this alternative was considered but eliminated from further consideration and is included in this section for comparison purposes only.

Refer to the end of this Section for the parameters used in the modeling.

Population Modeling Tables

Table 1: Population Sized in 11 years – Proposed Action

Trial	Population Sizes in 11 Years – Proposed Action		
	Minimum	Average	Maximum
Lowest Trial	95	142	243
Median Trial	134	175	265
Highest Trial	162	209	386

Table 2: Population Sized in 11 years – Alternative 1

Trial	Population Sizes in 11 Years – Alternative 1		
	Minimum	Average	Maximum
Lowest Trial	89	157	243
Median Trial	132	191	280
Highest Trial	159	229	365

Table 3: Population Sized in 11 years – No Action

Trial	Population Sizes in 11 Years – No Action		
	Minimum	Average	Maximum
Lowest Trial	244	470	828
Median Trial	261	748	1576
Highest Trial	357	1008	2265

Table 4: Population Sized in 11 years – Fertility control only

Trial	Population Sizes in 11 Years – No Action		
	Minimum	Average	Maximum
Lowest Trial	207	380	570
Median Trial	266	539	929
Highest Trial	343	745	1415

Table 4: Average Population Growth Rates in 11 Years

Trial	Proposed Action	Alternative 1	No Action	Fertility Control only
Lowest Trial	4.9	10.0	12.2	8.7
Median Trial	11.2	19.3	19.6	13.1
Highest Trial	18.8	26.2	23.6	16.3

Table 5: Gather Results in 11 Years – Proposed Action

Trial	Totals in 11 Years – Proposed Action		
	Gathered	Removed	Treated
Lowest	395	178	44
Median	493	302	62
Highest	630	463	82

Table 6: Gather Results in 11 Years – Alternative 1

Trial	Totals in 11 Years –Alternative 1		
	Gathered	Removed	Treated
Lowest	337	253	N/A
Median	504	392	N/A
Highest	708	557	N/A

Table 7: Gather Results in 11 Years – No Action

Trial	Totals in 11 Years –No Action		
	Gathered	Removed	Treated
Lowest	N/A	N/A	NA
Median	N/A	N/A	NA
Highest	N/A	N/A	NA

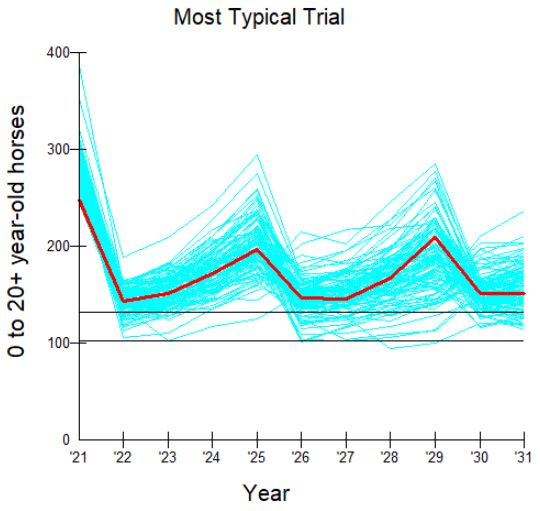
Table 8: Gather Results in 11 Years – Fertility Control Only

Trial	Totals in 11 Years –No Action		
	Gathered	Removed	Treated
Lowest	764	0	240
Median	1086	0	318
Highest	1463	0	412

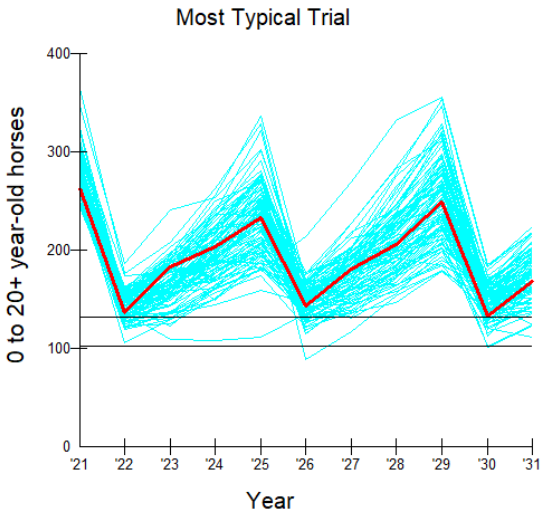
Table 9: Most Typical Trial Population by Year

Year	Proposed Action	Alternative 1	No Action	Fertility Control Only
Year 1 – 2021	163	184	165	183
Year 2 – 2022	149	145	196	250
Year 3 – 2023	149	172	245	246
Year 4 – 2024	164	193	258	269
Year 5 – 2025	162	226	293	270
Year 6 – 2026	155	140	277	345
Year 7 – 2027	160	148	359	344
Year 8 – 2028	188	188	417	357
Year 9 – 2029	199	222	517	439
Year 10 – 2030	145	140	673	520
Year 11 -- 2031	153	167	836	581
Average	163	175	385	346

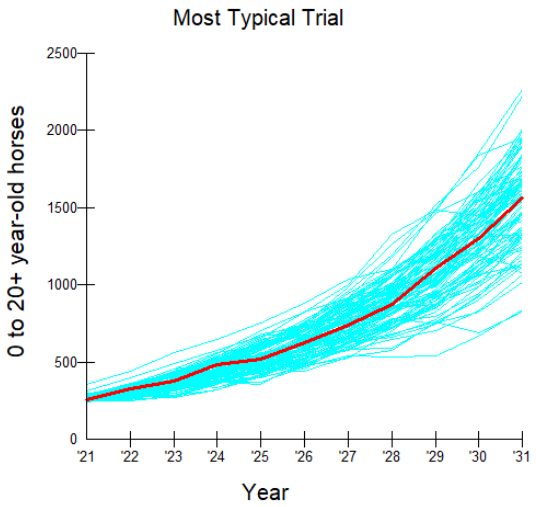
Population Modeling Graphics



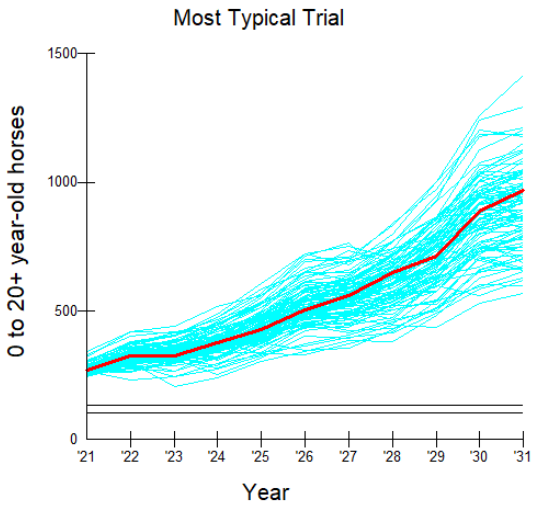
Proposed Action Most Typical Trial



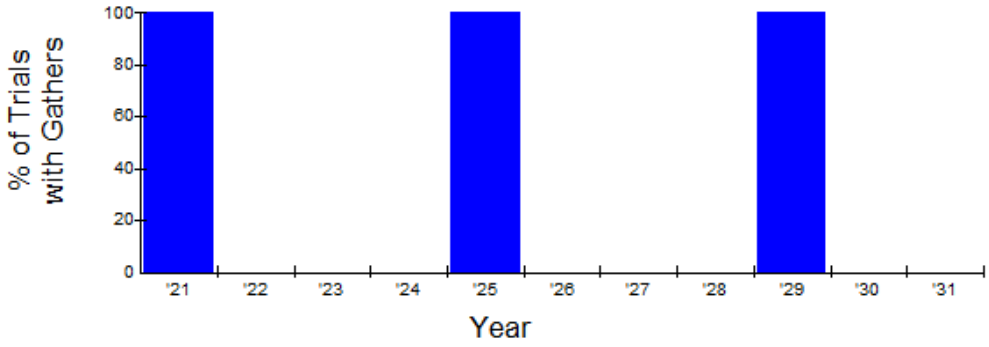
Alternative 1 Most Typical Trial



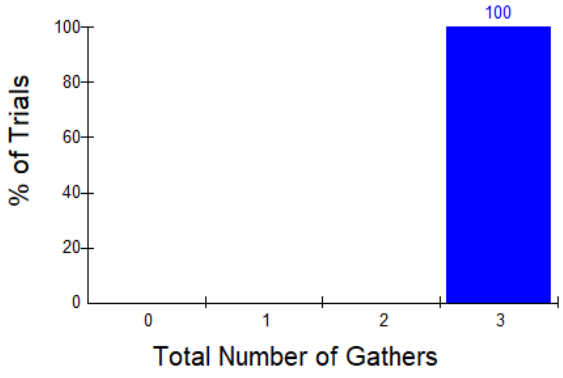
No Action Alternative Most Typical Trial



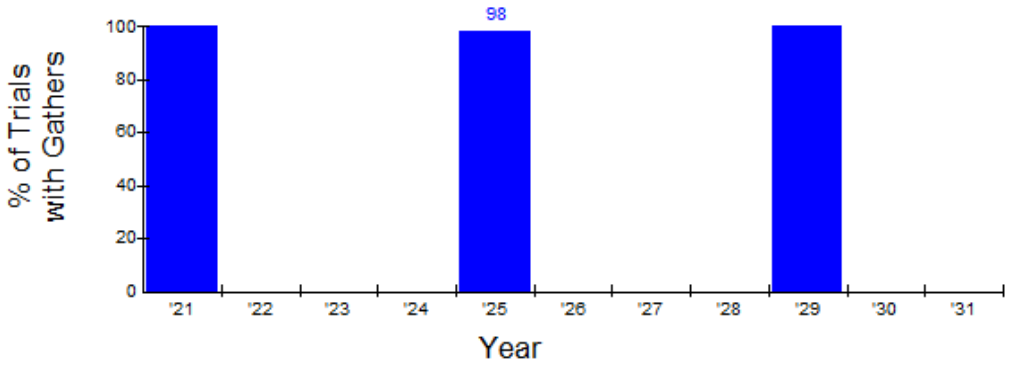
Fertility control only



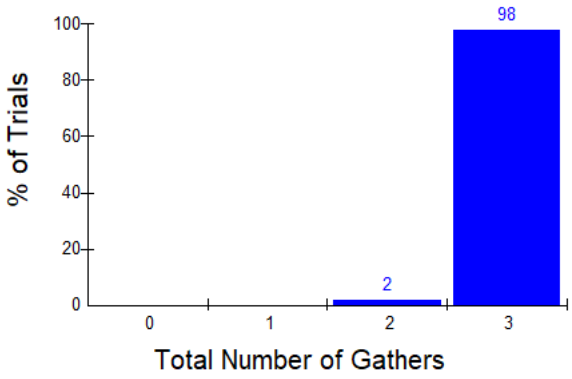
Proposed Action Gather schedule



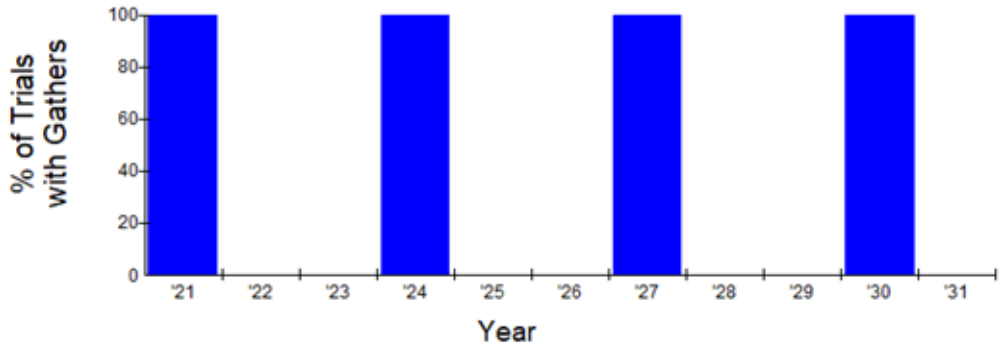
Proposed Action Gather total



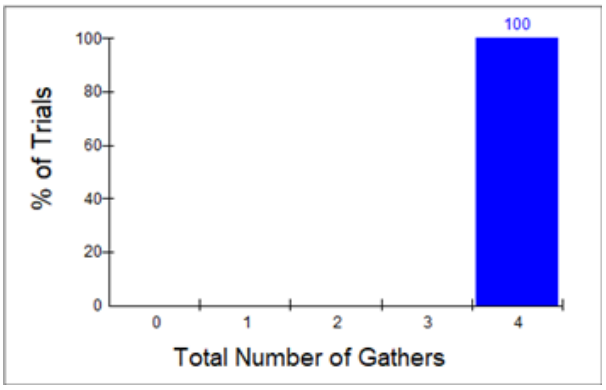
Alternative 1 Schedule of Gathers



Alternative 1 Gather total



Fertility Control Only schedule of gathers



Fertility control only gather total

Population Modeling Criteria

Population Data, Criteria, and Parameters Used for Population Modeling

All simulations used the survival probabilities and foaling rates that were supplied with the WinEquus data file.

- Initial age-sex distribution was scaled up to 350 horses, the estimated 2022 population.
- Fertility control parameters: Year 1—94%, Year 2—82%, Year 3—68%
- Initial Gather Year: 2022
- Gather interval: minimum interval of four years
- Gather for fertility treatment regardless of population size: No
- Continue to gather after reduction to treat females: Yes
- Percent of the population that can be gathered: 80%
- Minimum age for sanctuary horses: Not Applicable
- Foals are not included in the AML

Simulations were run for 10 years with 100 trials each. Modeling Parameter	Proposed Action: selective removal down to 79 horses with application of fertility control to mares	Alternative 2: Removal only (to 79 horses)	No Action: no removal or fertility control
Threshold population for gathers	132	132	N/A
Target post-gather population size	79	79	N/A
Gather for fertility control regardless of population size	No	No	No
Continue gathering after removals to treat additional females	Yes	No	No
Year 1 effectiveness of fertility control	94%	N/A	N/A
Year 2 effectiveness of fertility control	82%	N/A	N/A
Year 3 effectiveness of fertility control	68%	N/A	N/A

4.0 Gather Operations Standard Operating Procedures

Gathers would be conducted by utilizing contractors from the Wild Horse Gathers-Western States Contract, or BLM personnel. The following procedures for gathering and handling wild horses would apply whether a contractor or BLM personnel conduct a gather. For helicopter gathers conducted by BLM personnel, gather operations will be conducted in conformance with the *Wild Horse Aviation Management Handbook* (January 2009).

Prior to any gathering operation, the BLM will provide for a pre-gather evaluation of existing conditions in the gather area(s). The evaluation will include animal conditions, prevailing temperatures, drought conditions, soil conditions, road conditions, and a topographic map with wilderness boundaries, the location of fences, other physical barriers, and acceptable trap locations in relation to animal distribution. The evaluation will determine whether the proposed activities will necessitate the presence of a veterinarian during operations. If it is determined that a large number of animals may need to be euthanized or gather operations could be facilitated by a veterinarian, these services would be arranged before the gather would proceed. The contractor will be apprised of all conditions and will be given instructions regarding the gather and handling of animals to ensure their health and welfare is protected.

Trap sites and temporary holding sites will be located to reduce the likelihood of injury and stress to the animals, and to minimize potential damage to the natural resources of the area. These sites would be located on or near existing roads whenever possible.

The primary gather methods used in the performance of gather operations include:

1. Helicopter Drive Trapping. This gather method involves utilizing a helicopter to herd wild horses into a temporary trap.
2. Helicopter Assisted Roping. This gather method involves utilizing a helicopter to herd wild horses or burros to ropers.
3. Bait Trapping. This gather method involves utilizing bait (e.g., water or feed) to lure wild horses into a temporary trap.

The following procedures and stipulations will be followed to ensure the welfare, safety and humane treatment of wild horses in accordance with the provisions of 43 CFR 4700.

A. Gather Methods used in the Performance of Gather Contract Operations

1. The primary concern of the contractor is the safe and humane handling of all animals gathered. All gather attempts shall incorporate the following:

   All trap and holding facilities locations must be approved by the Contracting Officer's Representative (COR) and/or the Project Inspector (PI) prior to construction. The Contractor may also be required to change or move trap locations as determined by the COR/PI. All traps and holding facilities not located on public land must have prior written approval of the landowner.

2. The rate of movement and distance the animals travel shall not exceed limitations set by the COR who will consider terrain, physical barriers, access limitations, weather, extreme temperature (high and low), condition of the animals, urgency of the operation (animals facing drought, starvation, fire rehabilitation, etc.) and other factors. In consultation with the contractor the distance the animals travel will account for the different factors listed above and concerns with each HMA.

3. All traps, wings, and holding facilities shall be constructed, maintained and operated to handle the animals in a safe and humane manner and be in accordance with the following:

    a. Traps and holding facilities shall be constructed of portable panels, the top of which shall not be less than 72 inches high for horses and 60 inches for burros, and the bottom rail of which shall not be more than 12 inches from ground level. All traps and holding facilities shall be oval or round in design.

    b. All loading chute sides shall be a minimum of 6 feet high and shall be fully covered, plywood, metal without holes larger than 2"x4".

    c. All runways shall be a minimum of 30 feet long and a minimum of 6 feet high for horses, and 5 feet high for burros, and shall be covered with plywood, burlap, plastic snow fence or like material a minimum of 1 foot to 5 feet above ground level for burros and 1 foot to 6 feet for horses. The location of the government furnished portable fly chute to restrain, age, or provide additional care for the animals shall be placed in the runway in a manner as instructed by or in concurrence with the COR/PI.

    d. All crowding pens including the gates leading to the runways shall be covered with a material which prevents the animals from seeing out (plywood, burlap, plastic snow fence, etc.) and shall be covered a minimum of 1 foot to 5 feet above ground level for burros and 2 feet to 6 feet for horses

    e. All pens and runways used for the movement and handling of animals shall be connected with hinged self-locking or sliding gates.

4. No modification of existing fences will be made without authorization from the COR/PI. The Contractor shall be responsible for restoration of any fence modification which he has made.

5. When dust conditions occur within or adjacent to the trap or holding facility, the Contractor shall be required to wet down the ground with water.

6. Alternate pens, within the holding facility shall be furnished by the Contractor to separate mares or jennies with small foals, sick and injured animals, estrays or other animals the COR determines need to be housed in a separate pen from the other animals. Animals shall be sorted as to age, number, size, temperament, sex, and condition when in the holding facility so as to minimize, to the extent possible, injury due to fighting and trampling. Under normal conditions, the government will require that animals be restrained for the purpose of determining an animal's age, sex, or other necessary procedures. In these instances, a portable restraining chute may be necessary and will be provided by the government. Alternate pens shall be furnished by the Contractor to hold animals if the specific gathering requires that animals be released back into the gather area(s). In areas requiring one or more satellite traps, and where a centralized holding facility is utilized, the contractor may be required to provide additional holding pens to segregate animals transported from remote locations so they may be returned to their traditional ranges. Either segregation or temporary marking and later segregation will be at the discretion of the COR.

7. The Contractor shall provide animals held in the traps and/or holding facilities with a continuous supply of fresh clean water at a minimum rate of 10 gallons per animal per day. Animals held for 10 hours or more in the traps or holding facilities shall be provided good quality hay at the rate of

not less than two pounds of hay per 100 pounds of estimated body weight per day. The contractor will supply certified weed free hay if required by State, County, and Federal regulation.

a. An animal that is held at a temporary holding facility through the night is defined as a horse/burro feed day. An animal that is held for only a portion of a day and is shipped or released does not constitute a feed day.

8. It is the responsibility of the Contractor to provide security to prevent loss, injury or death of gathered animals until delivery to final destination.

9. The Contractor shall restrain sick or injured animals if treatment is necessary. The COR/PI will determine if animals must be euthanized and provide for the destruction of such animals. The Contractor may be required to humanely euthanize animals in the field and to dispose of the carcasses as directed by the COR/PI.

10. Animals shall be transported to their final destination from temporary holding facilities as quickly as possible after gather unless prior approval is granted by the COR for unusual circumstances. Animals to be released back into the HMA following gather operations may be held up to 21 days or as directed by the COR. Animals shall not be held in traps and/or temporary holding facilities on days when there is no work being conducted except as specified by the COR. The Contractor shall schedule shipments of animals to arrive at final destination between 7:00 a.m. and 4:00 p.m. No shipments shall be scheduled to arrive at final destination on Sunday and Federal holidays, unless prior approval has been obtained by the COR. Animals shall not be allowed to remain standing on trucks while not in transport for a combined period of greater than three (3) hours in any 24 hour period. Animals that are to be released back into the gather area may need to be transported back to the original trap site. This determination will be at the discretion of the COR/PI or Field Office horse specialist.

B. Gather Methods That May Be Used in the Performance of a Gather

1. Gather attempts may be accomplished by utilizing bait (feed, water, mineral licks) to lure animals into a temporary trap. If this gather method is selected, the following applies:

a. Finger gates shall not be constructed of materials such as "T" posts, sharpened willows, etc., that may be injurious to animals.

b. All trigger and/or trip gate devices must be approved by the COR/PI prior to gather of animals.

c. Traps shall be checked a minimum of once every 10 hours.

2. Gather attempts may be accomplished by utilizing a helicopter to drive animals into a temporary trap. If the contractor selects this method the following applies:

a. A minimum of two saddle-horses shall be immediately available at the trap site to accomplish roping if necessary. Roping shall be done as determined by the COR/PI. Under no circumstances shall animals be tied down for more than one half hour.

b. The contractor shall assure that foals shall not be left behind, or orphaned.

3. Gather attempts may be accomplished by utilizing a helicopter to drive animals to ropers. If the contractor, with the approval of the COR/PI, selects this method the following applies:

    a. Under no circumstances shall animals be tied down for more than one hour.

    b. The contractor shall assure that foals shall not be left behind, or orphaned.

    c. The rate of movement and distance the animals travel shall not exceed limitations set by the COR/PI who will consider terrain, physical barriers, weather, condition of the animals and other factors.

C. Use of Motorized Equipment

1. All motorized equipment employed in the transportation of gathered animals shall be in compliance with appropriate State and Federal laws and regulations applicable to the humane transportation of animals. The Contractor shall provide the COR/PI, if requested, with a current safety inspection (less than one year old) for all motorized equipment and tractor-trailers used to transport animals to final destination.

2. All motorized equipment, tractor-trailers, and stock trailers shall be in good repair, of adequate rated capacity, and operated so as to ensure that gathered animals are transported without undue risk or injury.

3. Only tractor-trailers or stock trailers with a covered top shall be allowed for transporting animals from trap site(s) to temporary holding facilities, and from temporary holding facilities to final destination(s). Sides or stock racks of all trailers used for transporting animals shall be a minimum height of 6 feet 6 inches from the floor. Single deck tractor-trailers 40 feet or longer shall have at least two (2) partition gates providing at least three (3) compartments within the trailer to separate animals. Tractor-trailers less than 40 feet shall have at least one partition gate providing at least two (2) compartments within the trailer to separate the animals. Compartments in all tractor-trailers shall be of equal size plus or minus 10 percent. Each partition shall be a minimum of 6 feet high and shall have a minimum 5 foot wide swinging gate. The use of double deck tractor-trailers is unacceptable and shall not be allowed.

4. All tractor-trailers used to transport animals to final destination(s) shall be equipped with at least one (1) door at the rear end of the trailer which is capable of sliding either horizontally or vertically. The rear door(s) of tractor-trailers and stock trailers must be capable of opening the full width of the trailer. Panels facing the inside of all trailers must be free of sharp edges or holes that could cause injury to the animals. The material facing the inside of all trailers must be strong enough so that the animals cannot push their hooves through the side. Final approval of tractor-trailers and stock trailers used to transport animals shall be held by the COR/PI.

5. Floors of tractor-trailers, stock trailers and loading chutes shall be covered and maintained with wood shavings to prevent the animals from slipping as much as possible during transport.

6. Animals to be loaded and transported in any trailer shall be as directed by the COR/PI and may include limitations on numbers according to age, size, sex, temperament and animal condition. The following minimum square feet per animal shall be allowed in all trailers:

    11 square feet per adult horse (1.4 linear foot in an 8 foot wide trailer);

8 square feet per adult burro (1.0 linear foot in an 8 foot wide trailer);
6 square feet per horse foal (.75 linear foot in an 8 foot wide trailer);
4 square feet per burro foal (.50 linear feet in an 8 foot wide trailer).

7. The COR/PI shall consider the condition and size of the animals, weather conditions, distance to be transported, or other factors when planning for the movement of gathered animals. The COR/PI shall provide for any brand and/or inspection services required for the gathered animals.

8. If the COR/PI determines that dust conditions are such that the animals could be endangered during transportation, the Contractor will be instructed to adjust speed.

D. Safety and Communications

1. The Contractor shall have the means to communicate with the COR/PI and all contractor personnel engaged in the gather of wild horses utilizing a VHF/FM Transceiver or VHF/FM portable Two-Way radio. If communications are ineffective the government will take steps necessary to protect the welfare of the animals.

    a. The proper operation, service and maintenance of all contractor furnished property is the responsibility of the Contractor. The BLM reserves the right to remove from service any contractor personnel or contractor furnished equipment which, in the opinion of the contracting officer or COR/PI violate contract rules, are unsafe or otherwise unsatisfactory. In this event, the Contractor will be notified in writing to furnish replacement personnel or equipment within 48 hours of notification. All such replacements must be approved in advance of operation by the Contracting Officer or his/her representative.

    b. The Contractor shall obtain the necessary FCC licenses for the radio system

    c. All accidents occurring during the performance of any task order shall be immediately reported to the COR/PI.

2. Should the contractor choose to utilize a helicopter the following will apply:

    a. The Contractor must operate in compliance with Federal Aviation Regulations, Part 91. Pilots provided by the Contractor shall comply with the Contractor's Federal Aviation Certificates, applicable regulations of the State in which the gather is located.

    b. Fueling operations shall not take place within 1,000 feet of animals.

E. Site Clearances

Gather sites and holding facilities would be located in previously disturbed areas whenever possible to minimize potential damage to the natural and cultural resources. Gather sites and temporary holding facilities would not be constructed on wetlands or riparian zones.

Prior to implementation of gather operations, gather sites and temporary holding facilities would be evaluated to determine their potential for containing cultural resources. All gather facilities (including gather sites, gather runways, blinds, holding facilities, camp locations, parking areas, staging areas, etc.) that would be located partially or totally in new locations (i.e. not at previously used gather locations) or in previously undisturbed areas would be inventoried by a BLM archaeologist or district archaeological

technician before initiation of the gather. A buffer of at least 50 meters would be maintained between gather facilities and any identified cultural resources. Once archaeological clearance has been obtained, the trap or temporary holding facility may be set up. Said clearance shall be arranged for by the COR, PI, or other BLM employees.

Gather sites and holding facilities would not be placed in known areas of Native American concern. No personnel working at gather sites may excavate, remove, damage, or otherwise alter or deface or attempt to excavate, remove, damage or otherwise alter or deface any archaeological resource located on public lands or Indian lands. The contractor would not disturb, alter, injure, or destroy any scientifically important paleontological remains; any historical or archaeological site, structure, building, grave, object or artifact; or any location having Native American traditional or spiritual significance within the project area or surrounding lands. The contractor would be responsible for ensuring that its employees, subcontractors or any others associated with the project do not collect artifacts and fossils, or damage or vandalize archaeological, historical or paleontological sites or the artifacts within them.

Should damage to cultural or paleontological resources occur during the period of gather due to the unauthorized, inadvertent or negligent actions of the contractor or any other project personnel, the contractor would be responsible for costs of rehabilitation or mitigation. Individuals involved in illegal activities may be subject to penalties under the Archaeological Resources Protection Act (ARPA).

F. Animal Characteristics and Behavior

Releases of wild horses would be near available water when possible. If the area is new to them, a short-term adjustment period may be required while the wild horses become familiar with the new area.

G. Public Participation

Opportunities for public viewing (i.e. media, interested public) of gather operations will be made available to the extent possible; however, the primary considerations will be to protect the health, safety and welfare of the animals being gathered and the personnel involved. The public must adhere to guidance from the on-site BLM representative. It is BLM policy that the public will not be allowed to come into direct contact with wild horses or burros being held in BLM facilities. Only authorized BLM personnel or contractors may enter the corrals or directly handle the animals. The general public may not enter the corrals or directly handle the animals at anytime or for any reason during BLM operations.

H. Responsibility and Lines of Communication

Contracting Officer's Representative/Project Inspector

Shawna Richardson, WH&B Specialist, Battle Mountain District
Brianna Brodowski, WH&B Specialist, Battle Mountain District, Tonopah Field Office
Ruth Thompson, NV WH&B Program Lead
Lance Brown, Humboldt Toiyabe National Forrest

The Contracting Officer's Representatives (CORs) and the project inspectors (PIs) have the direct responsibility to ensure the Contractor's compliance with the contract stipulations. The Schell Supervisory Natural Resource Specialist and the Schell Field Managers will take an active role to ensure the appropriate lines of communication are established between the field, Field Office, State Office, National Program Office, and BLM Holding Facility offices. All employees involved in the gathering operations will keep the best interests of the animals at the forefront at all times.

All publicity, formal public contact and inquiries will be handled through the Field Manager and/or the

Supervisory Natural Resource Specialist and Field Office Public Affairs. These individuals will be the primary contact and will coordinate with the COR/PI on any inquiries.

The COR will coordinate with the contractor and the BLM Corrals to ensure animals are being transported from the gather site in a safe and humane manner and are arriving in good condition.

The contract specifications require humane treatment and care of the animals during removal operations. These specifications are designed to minimize the risk of injury and death during and after gather of the animals. The specifications will be vigorously enforced.

Should the Contractor show negligence and/or not perform according to contract stipulations, he will be issued written instructions, stop work orders, or defaulted.

5.0 Wild Horse Gather Observation Protocol

BLM recognizes and respects the right of interested members of the public and the press to observe wild horse gather operations. At the same time, BLM must ensure the health and safety of the public, BLM's employees and contractors, and America's wild horses. Accordingly, the BLM developed these rules to maximize the opportunity for reasonable public access to the gather while ensuring that BLM's health and safety responsibilities are fulfilled. Failure to maintain safe distances from operations at the gather and temporary holding sites could result in members of the public inadvertently getting in the path of the wild horses or gather personnel, thereby placing themselves and others at risk, or causing stress and potential injury to the wild horses. The BLM and the contractor's helicopter pilot must comply with 14 CFR Part 91 of the Federal Aviation Regulations, which determines the minimum safe altitudes and distance people must be from the aircraft. To be in compliance with these regulations, the viewing location at the gather site and holding corrals must be approximately 500 feet from the operating location of the helicopter at all times. The viewing locations may vary depending on topography, terrain and other factors.

Daily Visitor Protocol

- A Wild Horse Gather Information Phone Line would be set up prior to the gather so the public can call for daily updates on gather information and statistics. Visitors are strongly encouraged to check the phone line the evening before they plan to attend the gather to confirm the gather and their tour of it is indeed taking place the next day as scheduled (weather, mechanical issues or other things may affect this) and to confirm the meeting location.

- Visitors must direct their questions/comments to either their designated BLM representative or the BLM spokesperson on site, and not engage other BLM/contractor staff and disrupt their gather duties/responsibilities - professional and respectful behavior is expected of all. BLM may make the BLM staff available during down times for a Q&A session on public outreach and education days. However, the contractor and its staff would not be available to answer questions or interact with visitors.

- Observers must provide their own 4-wheel drive high clearance vehicle, appropriate shoes, winter clothing, food and water. Observers are prohibited from riding in government and contractor vehicles and equipment.

- Gather operations may be suspended if bad weather conditions create unsafe flying conditions.

- BLM would establish one or more observation areas, in the immediate area of the gather and holding sites, to which individuals would be directed. These areas would be placed so as to maximize the opportunity for public observation while providing for a safe and effective wild horse gather. The utilization of such observation areas is necessary due to the use and presence of heavy equipment and aircraft in the gather operation and the critical need to allow BLM personnel and contractors to fully focus on attending to the needs of the wild horses while maintaining a safe environment for all involved. In addition, observation areas would be sited so as to protect the wild horses from being spooked, startled or impacted in a manner that results in increased stress.

- BLM would delineate observation areas with yellow caution tape (or a similar type of tape or ribbon).

- Visitors would be assigned to a specific BLM representative on public outreach and education days and must stay with that person at all times.

- Visitors are NOT permitted to walk around the gather site or temporary holding facility unaccompanied by their BLM representative.

- Observers are prohibited from climbing/trespassing onto or in the trucks, equipment or corrals, which is the private property of the contractor.

- When BLM is using a helicopter or other heavy equipment in close proximity to a designated observation area, members of the public may be asked to stay by their vehicle for some time before being directed to an observation area once the use of the helicopter or the heavy machinery is complete.

- When given the signal that the helicopter is close to the gather site bringing wild horses in, visitors must sit down in areas specified by BLM representatives and must not move or talk as the wild horses are guided into the corral.

- Individuals attempting to move outside a designated observation area would be requested to move back to the designated area or to leave the site. Failure to do so may result in citation or arrest. It is important to stay within the designated observation area to safely observe the wild horse gather.

- Observers would be polite, professional and respectful to BLM managers and staff and the contractor/employees. Visitors who do not cooperate and follow the rules would be escorted off the gather site by BLM law enforcement personnel and would be prohibited from participating in any subsequent observation days.

- BLM reserves the right to alter these rules based on changes in circumstances that may pose a risk to health, public safety or the safety of wild horses (such as weather, lightening, wildfire, etc.).

Public Outreach and Education Day

- The media and public are welcome to attend the gather any day and are encouraged to attend on public outreach and education days. On this day, BLM would have additional interpretive opportunities and staff available to answer questions.

- The number of public outreach and education days per week, and which days they are, would be determined prior to the gather and would be announced through a press release and on the website. Interested observers should RSVP ahead through the BLM-Battle Mountain.

- Office number (TBD). A meeting place would be set for each public outreach and education day and the RSVP list notified. BLM representatives would escort observers on public outreach and education days to and from the gather site and temporary holding facility.

6.0 Vegetation, Climate and Monitoring Data

Vegetation

The Little Fish Lake Valley JMA is located within the Central Nevada Basin and Range Major Land Resource Area (MLRA). This area is in the Great Basin Section of the Basin and Range Province of the Intermontane Plateaus. This MLRA supports saltbush-greasewood, big sagebrush, and pinyon-juniper woodland vegetation in the progression from the lowest to the highest elevation and precipitation. Shadscale, in association with bud sagebrush, spiny hopsage, ephedra, winterfat, fourwing saltbush, Indian ricegrass, squirreltail, and galleta, characterizes the saltbush-greasewood type. With an increase in moisture, plants associated with shadscale are replaced by needlegrasses, bluegrasses, bluebunch or beardless wheatgrass, basin wildrye, and forbs. Black greasewood and Nuttall saltbush are important on some sites. Big sagebrush and black sagebrush, which grows on soils that are shallow to an indurated pan or to bedrock, are dominant. In the pinyon-juniper woodland, bitterbrush, serviceberry, and snowberry grow in association with Utah juniper and singleleaf pinyon. The highest elevations support thickets of curl-leaf mountain mahogany and small amounts of mixed conifer forest with limber, bristlecone, or ponderosa pine, Douglas-fir, or white fir. On bottom lands, basin wildrye, creeping wildrye, alkali sacaton, wheatgrasses, bluegrasses, sedges, and rushes are typical. Black greasewood, rubber rabbitbrush, and big sagebrush grow on the drier sites. Inland saltgrass, alkali sacaton, black greasewood, rubber rabbitbrush, and big saltbush typify the vegetation on strongly saline-alkali soils (NRCS, 2006).

The Little Fish Lake Valley JMA is dominated by three naturally occurring ecological systems, as defined by the Southwest Regional Gap Analysis Project (SWREGap). Together, the Great Basin xeric mixed sagebrush shrublands, the intermountain basins big sagebrush shrublands, and Great Basin pinyon-juniper woodlands comprise approximately 90% of the total area. Some portions of the JMA have been altered as crested wheatgrass seedings.

Great Basin xeric mixed sagebrush shrublands comprise approximately 33% of the total area and occur on dry flats and plains, alluvial fans, rolling hills, rocky hillslopes, saddles and ridges at elevations between approximately 3,200 and 8,500 feet. Sites are dry, often exposed to desiccating winds, with typically shallow, rocky, non-saline soils. Within the JMA, these shrublands are dominated by black sage (mid and low elevations), low sage (higher elevation) and may be co-dominated by Wyoming big sagebrush or yellow rabbitbrush. Other shrubs that may be present include shadscale saltbush, Nevada ephedra, rubber rabbitbrush, spiny hopsage, Shockley's desert-thorn, budsage, greasewood, and horsebrush. The herbaceous layer is likely sparse and composed of perennial bunch grasses such as Indian ricegrass, squirreltail, or Sandberg bluegrass (Lowry, et al., 2005).

Intermountain basin big sagebrush shrublands comprise approximately 32% of the area on the broad basin between the mountain ranges, plains, and foothills between approximately 4,900 and 7,500 feet elevation. Soils are typically deep, well-drained and non-saline. These shrublands are dominated by basin big sagebrush and/or Wyoming big sagebrush. Scattered juniper, greasewood, and saltbushes may be present in some stands. Rabbitbrush co-dominates some disturbed stands. Perennial herbaceous components typically contribute less than 25% vegetative cover. Common graminoid species include Indian ricegrass, needleandthread grass, basin wildrye, galleta, or Sandberg bluegrass (Lowry, et al., 2005).

Great Basin pinyon-juniper woodlands comprise approximately 25% of the JMA. This ecological system occurs on the dry mountain ranges and foothills, at elevations ranging from 5,250 to 8,500 feet. These woodlands occur on warm, dry sites on mountain slopes, mesas, plateaus, and ridges. Severe climatic events occurring during the growing season, such as frosts and drought, are thought to limit the distribution of pinyon-juniper woodlands to relatively narrow altitudinal belts on mountainsides.

Woodlands dominated by a mix of pinyon and juniper, pure or nearly pure occurrences of pinyon, or woodlands dominated solely by juniper comprise this system. Curl-leaf mountain mahogany is a common associate. Understory layers are variable. Associated species include shrubs such as Greenleaf manzanita, low sage, black sage, big sagebrush, or littleleaf mountain mahogany. Common herbaceous component includes bunch grasses needleandthread and basin wildrye (Lowry, et al., 2005).

Climate

The climate associated with the Little Fish Lake JMA is typical of the Basin and Range Ecological Region and characterized as having generally hot/dry summers and cold/wet winters. However, the past 10 years have frequently seen warmer than average summers and drier than average winters. Annual total precipitation can be highly variable with a few years receiving above average precipitation while most year receiving below average precipitation. The 30 year average annual precipitation at the JMA is 7.83 inches, with 1998 being the wettest and 2020 being the driest.

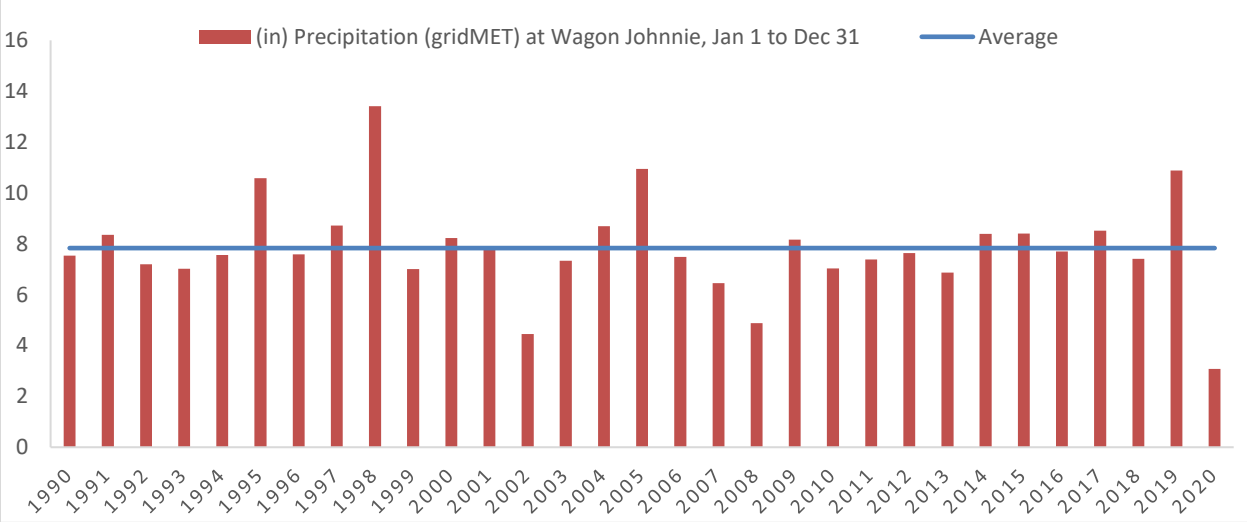
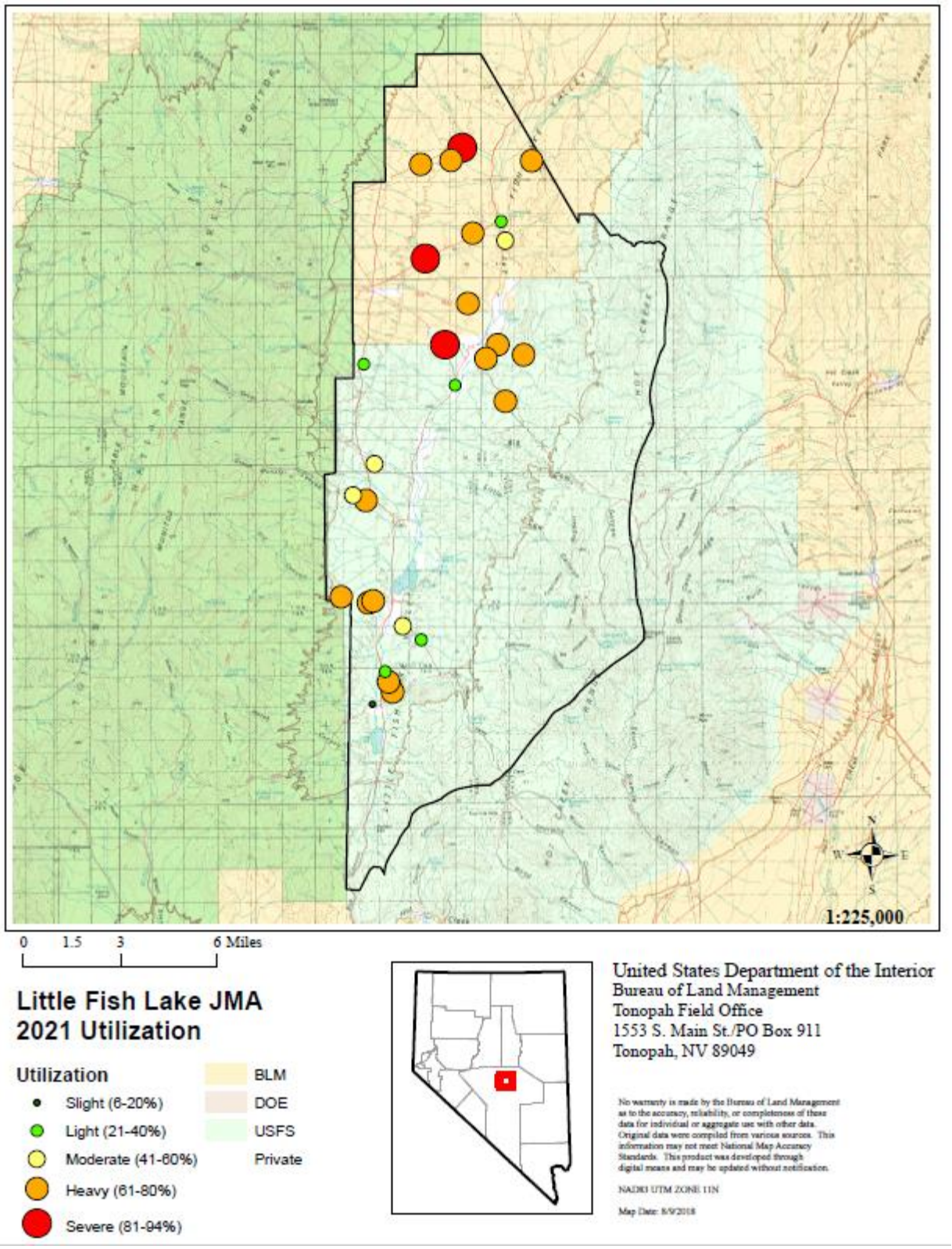


Table 1. 30 year average precipitation for the Wagon Johnnie BLM Allotment (Climate Engine).

Monitoring Data

Utilization

Key area utilization was conducted at 28 plots in December 2021 by Tonopah BLM staff and Intermountain Range Consultants, Inc., retained by Colvin & Son, LLC. Key species use ranged from negligible to severe use at key areas, with some key areas lacking key species entirely (see map below). At many key areas, remaining stubble height of key forage species that were present was around one inch. Utilization on winterfat and crested wheatgrass is severe and repeated, both species show signs of reduced vigor and reproductive capability are severely reduced and continued use by wild horses may impact the species continued occurrence on the landscape.



Map 3. 2021 Utilization for the Little Fish Lake HMA.

In summary, based on available monitoring data an excess number of wild horses in the Little Fish Lake JMA are contributing to the over utilization of key species such as Indian ricegrass, winterfat, and crested wheatgrass. Current vegetative conditions in the JMA, such as reduced frequency of key species; reduced cover of key grass species; and a transition to a less desirable shrub dominated plant community has forced wild horses onto private property inside the JMA. Impacts to private property include damage to fences, water developments, and degradation of private meadows and springs.

Photo 1. Heavy trailing in the Little Fish Lake JMA March 2021.

Photo 2. Heavy trailing in the Little Fish Lake JMA March 2021.

Photo 3. Heavy wild horse trailing and stud piles west of Sevenmile Spring.

Photo 4. Trailing to Clear Creek, November 2021.

Photo 5. Damaged fence along Clear Creek.

Photo 6. Damaged fence along Clear Creek.

Springs and Riparian Areas

At numerous springs, riparian, wetlands areas in the JMA, repeated use by wild horses has impacted the functionality over the years. The BLM has constructed exclosures at the Sevenmile Spring and Clear Creek to protect those sensitive areas from livestock and wild horse use, but the fences have been repeatedly damaged by wild horses. BLM staff visited these areas November 2021 and observed fence damage, heavy trailing, and horse use contributing to streambank erosion and reduced vigor of riparian plants (see appendix A).

Photo 7. Willows along clear creek showing reduced vigor due to likely herbivory by horses.

Photo 8. Trailing and erosion along banks of Clear Creek.

Photo 9. Trailing and erosion along banks of Clear Creek.

Photo 10. Grazing exclosure near Sevenmile Spring showing grazed vs. ungrazed plants.


Photos 11 and 12. Heavy trailing near Sevenmile spring.


Photos 13 and 14. Fence damage along Sevenmile Spring.


Photos 15 and 16. Area outside of Sevenmile spring showing heavy horse use

Photo 17. Sevenmile Spring, 2021.

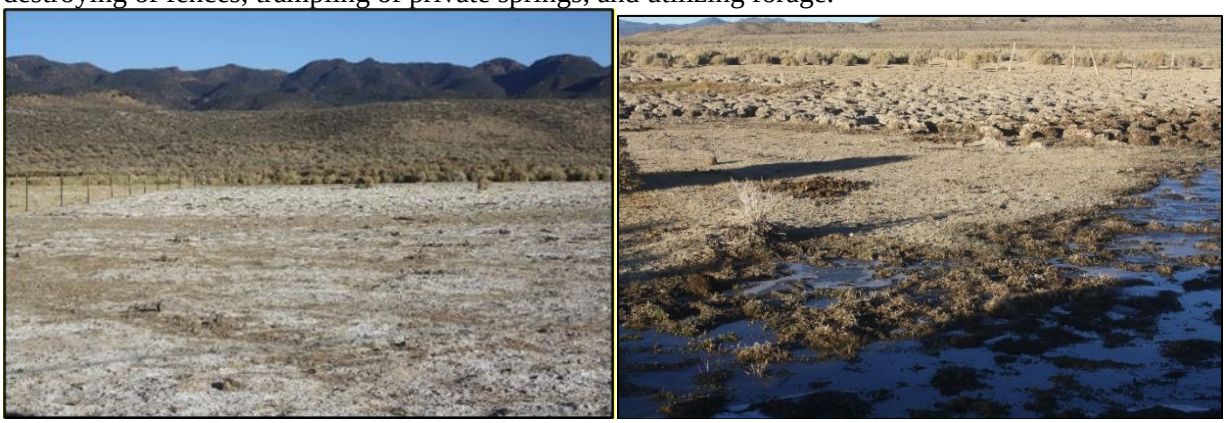
Photo 28. Sevenmile Spring outflow, 2021.

Photo 38. Anderson Spring Meadow, stud piles.

Photo 49. Anderson Spring Meadow, terracing from animal hooves.

Private Property Impacts

With limited vegetive and water resources located on public lands in the JMA, excess wild horse have ventured on private property in search of resources. Impacts from wild horses to private property include destroying of fences, trampling of private springs, and utilizing forage.



Photos 20 and 21. Horse utilization of privately owned and fenced section of Sevenmile Spring.

Photo 22. Wild horses just west of Colvin & Son, LLC's private property. These are resident horses that enter the private property in the evenings by breaking through the fence between BLM and private

Photo 23. Fenceline and gate on east side of Colvin & Son LLC's private property. Horses continuously breaking down the gate between USFS and private property. Property owner using cans and bottles on wire to deter horses from breaking through.

Photo 24. Fenceline damaged by wild horses on USFS and private property line.

Photo 25. Fenceline between USFS and private where property owner has had to reinforce fence with extra cedar stays to fix broken fence and to keep wild horses from breaking through the fence.

Photo 26. Gate between USFS and private that is continually broken and fixed by private landowner.

Photo 27. USFS land, heavy trailing coming from USFS down towards the private property.

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7.0 Dietary Overlap

Diet/dietary Overlap with Other Species

Wild horse populations above AML compete for forage, water, and cover allocated to wildlife and livestock. Over populations of wild horses impact riparian areas with increased trailing, vegetative use, and trampling. Wild horses in such situations will drive away livestock and native ungulates from watering and feeding areas (Miller 1981).

Numerous studies identify dietary overlap of preferred forage species and habitat preference between horses, cattle, and wildlife species in the Great Basin ecosystems for all season (Ganskopp 1983; Gandskopp et al. 1986, 1987; McInnis 1984; McInnis 1987; Smith et al 1982; Vavra and Sneva 1987). A strong potential exists for exploitative competition between horses and cattle under conditions of limited forage (water and space) availability (McInnis et al. 1987).

Although horses and cattle are often compared as grazers, horses can be more destructive to the range than cattle due to their differing digestive systems and grazing habits. The dietary overlap between wild horses and cattle is much higher than with wildlife, and averages between 60 and 80% (Hubbard and Hansen 1976, Hansen et al. 1977, Hanley 1982, Krysl et al. 1984, McInnis and Vavra 1987). Horses are cecal digesters while most other ungulates including cattle, pronghorn, and others are ruminants (Hanley and Hanley 1982, Beever 2003). Cecal digesters do not ruminate, or have to regurgitate and repeat the cycle of chewing until edible particles of plant fiber are small enough for their digestive system. Ruminants, especially cattle, must graze selectively, searching out digestible tissue (Olsen and Hansen 1977). Horses, however, are one of the least selective grazers in the West because they can consume high fiber foods and digest larger food fragments (Hanley and Hanley 1982, Beever 2003). Because horses have a cecal digestive system and can cover longer distances than domestic ruminants, wild horses can remain in good health under forage conditions fatal to domestic ruminants (Holechek 1989).

Wild horses can exploit the high cellulose of graminoids, or grasses, which have been observed to make up over 88% of their diet (McInnis and Vavra 1987, Hanley 1982). However, this lower quality diet requires that horses consume 20-65% more forage than a cow of equal body mass (Hanley 1982, Menard et al. 2002). With more flexible lips and upper front incisors, both features that cattle do not have, wild horses trim vegetation more closely to the ground (Symanski 1994, Menard et al. 2002, Beever 2003). As a result, areas grazed by horses may retain fewer plant species and may be subject to higher utilization levels than areas grazed by cattle or other ungulates. A potential benefit of a horse's digestive system may come from seeds passing through system without being digested but the benefit is likely minimal when compared to the overall impact wild horse grazing has on vegetation in general.

Competition from a large dominant species may drive niche partitioning of other species (Carothers and Jaksi, 1984; Ziv et al., 1993; Schuette et al., 2013). The study found that during times of greatest physiological stress (increased temperature, decreased precipitation), horses monopolized access to water sources where they were present up to 73% of the day, leaving limited time for other species. The potential for an exotic species, such as the wild horses, to outcompete native species for a limited communal resourced during peak need raises concern for native communities in water-limited environments (Hall et al. 2016).

8.0 Literature Reviews of PZP, GonaCon, and Intrauterine Devices (IUDs)

8.1 Porcine Zona Pellucida (PZP) and GonaCon Vaccines

Various forms of fertility control can be used in wild horses and wild burros, with the goals of maintaining herds at or near AML, reducing fertility rates, and reducing the frequency of gathers and removals. The WFRHBA of 1971 specifically provides for contraception and sterilization (16 U.S.C. 1333 section 3.b.1). Fertility control measures have been shown to be a cost-effective and humane treatment to slow increases in wild horse populations or, when used in combination with gathers, to reduce horse population size (Bartholow 2004, de Seve and Boyles-Griffin 2013, Fonner and Bohara 2017). Although fertility control treatments may be associated with a number of potential physiological, behavioral, demographic, and genetic effects, those impacts are generally minor and transient, do not prevent overall maintenance of a self-sustaining population, and do not generally outweigh the potential benefits of using contraceptive treatments in situations where it is a management goal to reduce population growth rates (Garrott and Oli 2013).

An extensive body of peer-reviewed scientific literature details the impacts of fertility control methods on wild horses and burros. No finding of excess animals is required for BLM to pursue contraception in wild horses or wild burros, but NEPA analysis has been required. This review focuses on peer-reviewed scientific literature. The summary that follows first examines effects of fertility control vaccine use in mares, then of sex ratio manipulation. This review does not examine effects of spaying and neutering. Cited studies are generally limited to those involving horses and burros, except where including studies on other species helps in making inferences about physiological or behavioral questions not yet addressed in horses or burros specifically. While most studies reviewed here refer to horses, burros are extremely similar in terms of physiology, such that expected effects are comparable, except where differences between the species are noted.

On the whole, the identified impacts are generally transient and affect primarily the individuals treated. Fertility control that affects individual horses and burros does not prevent BLM from ensuring that there will be self-sustaining populations of wild horses and burros in single herd management areas (HMAs), in complexes of HMAs, and at regional scales of multiple HMAs and complexes. Under the WFRHBA of 1971, BLM is charged with maintaining self-reproducing populations of wild horses and burros. The National Academies of Sciences (2013) encouraged BLM to manage wild horses and burros at the spatial scale of “metapopulations” – that is, across multiple HMAs and complexes in a region. In fact, many HMAs have historical and ongoing genetic and demographic connections with other HMAs, and BLM routinely moves animals from one to another to improve local herd traits and maintain high genetic diversity. The NAS report (2013) includes information (pairwise genetic 'fixation index' values for sampled WH&B herds) confirming that WH&B in the vast majority of HMAs are genetically similar to animals in multiple other HMAs.

All fertility control methods affect the behavior and physiology of treated animals (NAS 2013), and are associated with potential risks and benefits, including effects of handling, frequency of handling, physiological effects, behavioral effects, and reduced population growth rates (Hampton et al. 2015). Contraception alone does not remove excess horses from an HMA’s population, so one or more gathers are usually needed in order to bring the herd down to a level close to AML. Horses are long-lived, potentially reaching 20 years of age or more in the wild. Except in cases where extremely high fractions of mares are rendered infertile over long time periods of (i.e., 10 or more years), fertility control methods such as immunocontraceptive vaccines and sex ratio manipulation are not very effective at reducing population growth rates to the point where births equal deaths in a herd. However, even more modest fertility control activities can reduce the frequency of horse gather activities, and costs to taxpayers.

Bartholow (2007) concluded that the application of 2-year or 3-year contraceptives to wild mares could reduce operational costs in a project area by 12-20%, or up to 30% in carefully planned population management programs. Because applying contraception to horses requires capturing and handling, the risks and costs associated with capture and handling of horses may be comparable to those of gathering for removal, but with expectedly lower adoption and long-term holding costs. Population growth suppression becomes less expensive if fertility control is long-lasting (Hobbs et al. 2000).

In the context of BLM wild horse and burro management, fertility control vaccines and sex ratio manipulation rely on reducing the number of reproducing females. Taking into consideration available literature on the subject, the National Academies of Sciences concluded in their 2013 report that forms of fertility control vaccines were two of the three 'most promising' available methods for contraception in wild horses and burros (NAS 2013). That report also noted that sex ratio manipulations where herds have approximately 60% males and 40% females can expect lower annual growth rates, simply as a result of having a lower number of reproducing females.

Fertility Control Vaccines

Fertility control vaccines (also known as (immunocontraceptives) meet BLM requirements for safety to mares and the environment (EPA 2009a, 2012). Because they work by causing an immune response in treated animals, there is no risk of hormones or toxins being taken into the food chain when a treated mare dies. The BLM and other land managers have mainly used three fertility control vaccine formulations for fertility control of wild horse mares on the range: ZonaStat-H, PZP-22, and GonaCon-Equine. As other formulations become available they may be applied in the future.

In any vaccine, the antigen is the stimulant to which the body responds by making antigen-specific antibodies. Those antibodies then signal to the body that a foreign molecule is present, initiating an immune response that removes the molecule or cell. Adjuvants are additional substances that are included in vaccines to elevate the level of immune response. Adjuvants help to incite recruitment of lymphocytes and other immune cells which foster a long-lasting immune response that is specific to the antigen.

Liquid emulsion vaccines can be injected by hand or remotely administered in the field using a pneumatic dart (Roelle and Ransom 2009, Rutberg et al. 2017, McCann et al. 2017) in cases where mares are relatively approachable. Use of remotely delivered (dart-delivered) vaccine is generally limited to populations where individual animals can be accurately identified and repeatedly approached within 50 m (BLM 2010). Booster doses can be safely administered by hand or by dart. Even with repeated booster treatments of the vaccines, it is expected that most mares would eventually return to fertility, though some individual mares treated repeatedly may remain infertile. Once the herd size in a project area is at AML and population growth seems to be stabilized, BLM can make adaptive determinations as to the required frequency of new and booster treatments.

BLM has followed SOPs for fertility control vaccine application (BLM IM 2009-090). Herds selected for fertility control vaccine use should have annual growth rates over 5%, have a herd size over 50 animals, and have a target rate of treatment of between 50% and 90% of female wild horses or burros. The IM requires that treated mares be identifiable via a visible freeze brand or individual color markings, so that their vaccination history can be known. The IM calls for follow-up population surveys to determine the realized annual growth rate in herds treated with fertility control vaccines.

Vaccine Formulations: Porcine Zona Pellucida (PZP)

PZP vaccines have been used on dozens of horse herds by the National Park Service, US Forest Service, Bureau of Land Management, and Native American tribes and PZP vaccine use is approved for free-ranging wild and feral horse herds in the United States (EPA 2012). PZP use can reduce or eliminate the need for gathers and removals, if very high fractions of mares are treated over a very long time period

(Turner et al. 1997). PZP vaccines have been used extensively in wild horses (NAS 2013), and in feral burros on Caribbean islands (Turner et al. 1996, French et al. 2017). PZP vaccine formulations are produced as ZonaStat-H, an EPA-registered commercial product (EPA 2012, SCC 2015), as PZP-22, which is a formulation of PZP in polymer pellets that can lead to a longer immune response (Turner et al. 2002, Rutberg et al. 2017), and as Spayvac, where the PZP protein is enveloped in liposomes (Killian et al. 2008, Roelle et al. 2017, Bechert and Fraker 2018). 'Native' PZP proteins can be purified from pig ovaries (Liu et al. 1989). Recombinant ZP proteins may be produced with molecular techniques (Gupta and Minhas 2017, Joonè et al. 2017a, Nolan et al. 2018a).

When advisories on the product label (EPA 2015) are followed, the product is safe for users and the environment (EPA 2012). In keeping with the EPA registration for ZonaStat-H (EPA 2012; reg. no. 86833-1), certification through the Science and Conservation Center in Billings Montana is required to apply that vaccine to equids.

For maximum effectiveness, PZP is administered within the December to February timeframe. When applying ZonaStat-H, first the primer with modified Freund's Complete adjuvant is given and then the booster with Freund's Incomplete adjuvant is given 2-6 weeks later. Preferably, the timing of the booster dose is at least 1-2 weeks prior to the onset of breeding activity. Following the initial 2 inoculations, only annual boosters are required. For the PZP-22 formulation, each released mare would receive a single dose of the two-year PZP contraceptive vaccine at the same time as a dose of the liquid PZP vaccine with modified Freund's Complete adjuvant. The pellets are applied to the mare with a large gauge needle and jab-stick into the hip. Although PZP-22 pellets have been delivered via darting in trial studies (Rutberg et al 2017, Carey et al. 2019), BLM does not plan to use darting for PZP-22 delivery until there is more demonstration that PZP-22 can be reliably delivered via dart.

Vaccine Formulations: Gonadotropin Releasing Hormone (GnRH)
GonaCon (which is produced under the trade name GonaCon-Equine for use in feral horses and burros) is approved for use by authorized federal, state, tribal, public and private personnel, for application to free-ranging wild horse and burro herds in the United States (EPA 2013, 2015). GonaCon has been used on feral horses in Theodore Roosevelt National Park and on wild horses administered by BLM (BLM 2015). GonaCon has been produced by USDA-APHIS (Fort Collins, Colorado) in several different formulations, the history of which is reviewed by Miller et al. (2013). GonaCon vaccines present the recipient with hundreds of copies of GnRH as peptides on the surface of a linked protein that is naturally antigenic because it comes from invertebrate hemocyanin (Miller et al 2013). Early GonaCon formulations linked many copies of GnRH to a protein from the keyhole limpet (GonaCon-KHL), but more recently produced formulations where the GnRH antigen is linked to a protein from the blue mussel (GonaCon-B) proved less expensive and more effective (Miller et al. 2008). GonaCon-Equine is in the category of GonaCon-B vaccines.

As with other contraceptives applied to wild horses, the long-term goal of GonaCon-Equine use is to reduce or eliminate the need for gathers and removals (NAS 2013). GonaCon-Equine contraceptive vaccine is an EPA-approved pesticide (EPA, 2009a) that is relatively inexpensive, meets BLM requirements for safety to mares and the environment, and is produced in a USDA-APHIS laboratory. GonaCon is a pharmaceutical-grade vaccine, including aseptic manufacturing technique to deliver a sterile vaccine product (Miller et al. 2013). If stored at 4° C, the shelf life is 6 months (Miller et al 2013).

Miller et al. (2013) reviewed the vaccine environmental safety and toxicity. When advisories on the product label (EPA 2015) are followed, the product is safe for users and the environment (EPA 2009b). EPA waived a number of tests prior to registering the vaccine, because GonaCon was deemed to pose low risks to the environment, so long as the product label is followed (Wang-Cahill et al., *in press*).

GonaCon-Equine can safely be reapplied as necessary to control the population growth rate; booster dose effects may lead to increased effectiveness of contraception, which is generally the intent. Even after booster treatment of GonaCon-Equine, it is expected that most, if not all, mares would return to fertility at some point. Although the exact timing for the return to fertility in mares boosted more than once with GonaCon-Equine has not been quantified, a prolonged return to fertility would be consistent with the desired effect of using GonaCon (e.g., effective contraception).

The adjuvant used in GonaCon, Adjuvac, generally leads to a milder reaction than Freund's Complete Adjuvant (Powers et al. 2011). Adjuvac contains a small number of killed *Mycobacterium avium* cells (Miller et al. 2008, Miller et al. 2013). The antigen and adjuvant are emulsified in mineral oil, such that they are not all presented to the immune system right after injection. It is thought that the mineral oil emulsion leads to a 'depot effect' that is associated with slow or sustained release of the antigen, and a resulting longer-lasting immune response (Miller et al. 2013). Miller et al. (2008, 2013) have speculated that, in cases where memory-B leukocytes are protected in immune complexes in the lymphatic system, it can lead to years of immune response. Increased doses of vaccine may lead to stronger immune reactions, but only to a certain point; when Yoder and Miller (2010) tested varying doses of GonaCon in prairie dogs, antibody responses to the 200μg and 400μg doses were equal to each other but were both higher than in response to a 100μg dose.

Direct Effects: PZP Vaccines

The historically accepted hypothesis explaining PZP vaccine effectiveness posits that when injected as an antigen in vaccines, PZP causes the mare's immune system to produce antibodies that are specific to zona pellucida proteins on the surface of that mare's eggs. The antibodies bind to the mare's eggs surface proteins (Liu et al. 1989), and effectively block sperm binding and fertilization (Zoo Montana, 2000). Because treated mares do not become pregnant but other ovarian functions remain generally unchanged, PZP can cause a mare to continue having regular estrus cycles throughout the breeding season. More recent observations support a complementary hypothesis, which posits that PZP vaccination causes reductions in ovary size and function (Mask et al. 2015, Joonè et al. 2017b, Joonè et al. 2017c, Nolan et al. 2018b, 2018c). PZP vaccines do not appear to interact with other organ systems, as antibodies specific to PZP protein do not crossreact with tissues outside of the reproductive system (Barber and Fayrer-Hosken 2000).

Research has demonstrated that contraceptive efficacy of an injected liquid PZP vaccine, such as ZonaStat-H, is approximately 90% or more for mares treated twice in the first year (Turner and Kirkpatrick 2002, Turner et al. 2008). The highest success for fertility control has been reported when the vaccine has been applied November through February. High contraceptive rates of 90% or more can be maintained in horses that are given a booster dose annually (Kirkpatrick et al. 1992). Approximately 60% to 85% of mares are successfully contracepted for one year when treated simultaneously with a liquid primer and PZP-22 pellets (Rutberg et al. 2017, Carey et al. 2019). Application of PZP for fertility control would reduce fertility in a large percentage of mares for at least one year (Ransom et al. 2011). The contraceptive result for a single application of the liquid PZP vaccine primer dose along with PZP vaccine pellets (PZP-22), based on winter applications, can be expected to fall in the approximate efficacy ranges as follows (based on figure 2 in Rutberg et al. 2017). Below, the approximate efficacy is measured as the relative decrease in foaling rate for treated mares, compared to control mares:

Year 1	Year 2	Year 3
0 (developing fetuses come to term)	~30-75%	~20-50%

If mares that have been treated with PZP-22 vaccine pellets subsequently receive a booster dose of either the liquid PZP vaccine or the PZP-22 vaccine pellets, the subsequent contraceptive effect is apparently

more pronounced and long-lasting. The approximate efficacy following a booster dose can be expected to be in the following ranges (based on figure 3 in Rutberg et al. 2017).

Year 1	Year 2	Year 3	Year 4
0 (developing fetuses come to term)	~50-90%	~55-75%	~40-75%

The fraction of mares treated in a herd can have a large effect on the realized change in growth rate due to PZP contraception, with an extremely high portion of mares required over many years to be treated to totally prevent population-level growth (e.g., Turner and Kirkpatrick 2002). Gather efficiency does not usually exceed 85% via helicopter, and may be less with bait and water trapping, so there will almost always be a portion of the female population uncaptured that is not treated in any given year. Additionally, some mares may not respond to the fertility control vaccine, but instead will continue to foal normally.

Direct Effects: GnRH Vaccines

GonaCon-Equine is one of several vaccines that have been engineered to create an immune response to the gonadotropin releasing hormone peptide (GnRH). GnRH is a small peptide that plays an important role in signaling the production of other hormones involved in reproduction in both sexes. When combined with an adjuvant, a GnRH vaccine stimulates a persistent immune response resulting in prolonged antibody production against GnRH, the carrier protein, and the adjuvant (Miller et al., 2008). The most direct result of successful GnRH vaccination is that it has the effect of decreasing the level of GnRH signaling in the body, as evidenced by a drop in luteinizing hormone levels, and a cessation of ovulation.

GnRH is highly conserved across mammalian taxa, so some inferences about the mechanism and effects of GonaCon-Equine in horses can be made from studies that used different anti-GnRH vaccines, in horses and other taxa. Other commercially available anti-GnRH vaccines include: Improvac (Imboden et al. 2006, Botha et al. 2008, Janett et al. 2009a, Janett et al. 2009b, Schulman et al. 2013, Dalmau et al. 2015, Nolan et al. 2018c), made in South Africa; Equity (Elhay et al. 2007), made in Australia; Improvest, for use in swine (Bohrer et al. 2014); Repro-BLOC (Boedeker et al. 2011); and Bopriva, for use in cows (Balet et al. 2014). Of these, GonaCon-Equine, Improvac, and Equity are specifically intended for horses. Other anti-GnRH vaccine formulations have also been tested, but did not become trademarked products (e.g., Goodloe 1991, Dalin et al 2002, Stout et al. 2003, Donovan et al. 2013, Schaut et al. 2018, Yao et al. 2018). The effectiveness and side-effects of these various anti-GnRH vaccines may not be the same as would be expected from GonaCon-Equine use in horses. Results could differ as a result of differences in the preparation of the GnRH antigen, and the choice of adjuvant used to stimulate the immune response. For some formulations of anti-GnRH vaccines, a booster dose is required to elicit a contraceptive response, though GonaCon can cause short-term contraception in a fraction of treated animals from one dose (Powers et al. 2011, Gionfriddo et al. 2011a, Baker et al. 2013, Miller et al 2013).

GonaCon can provide multiple years of infertility in several wild ungulate species, including horses (Killian et al., 2008; Gray et al., 2010). The lack of estrus cycling that results from successful GonaCon vaccination has been compared to typical winter period of anoestrus in open mares. As anti-GnRH antibodies decline over time, concentrations of available endogenous GnRH increase and treated animals usually regain fertility (Power et al., 2011).

Females that are successfully contracepted by GnRH vaccination enter a state similar to anestrus, have a lack of or incomplete follicle maturation, and no ovarian cycling (Botha et al. 2008, Nolan et al. 2018c). A leading hypothesis is that anti-GnRH antibodies bind GnRH in the hypothalamus – pituitary 'portal

vessels,' preventing GnRH from binding to GnRH-specific binding sites on gonadotroph cells in the pituitary, thereby limiting the production of gonadotropin hormones, particularly luteinizing hormone (LH) and, to a lesser degree, follicle-stimulating hormone (FSH) (Powers et al. 2011, NAS 2013). This reduction in LH (and FSH), and a corresponding lack of ovulation, has been measured in response to treatment with anti-GnRH vaccines (Boedeker et al. 2011, Garza et al. 1986).

Females successfully treated with anti-GnRH vaccines have reduced progesterone levels (Garza et al. 1986, Stout et al. 2003, Imboden et al. 2006, Elhay 2007, Botha et al. 2008, Killian et al. 2008, Miller et al. 2008, Janett et al. 2009, Schulman et al. 2013, Balet et al 2014, Dalmau et al. 2015) and β-17 estradiol levels (Elhay et al. 2007), but no great decrease in estrogen levels (Balet et al. 2014). Reductions in progesterone do not occur immediately after the primer dose, but can take several weeks or months to develop (Elhay et al. 2007, Botha et al. 2008, Schulman et al. 2013, Dalmau et al. 2015). This indicates that ovulation is not occurring and corpora lutea, formed from post-ovulation follicular tissue, are not being established.

Antibody titer measurements are proximate measures of the antibody concentration in the blood specific to a given antigen. Anti-GnRH titers generally correlate with a suppressed reproduction system (Gionfriddo et al. 2011a, Powers et al. 2011). Various studies have attempted to identify a relationship between anti-GnRH titer levels and infertility, but that relationship has not been universally predictable or consistent. The time length that titer levels stay high appears to correlate with the length of suppressed reproduction (Dalin et al. 2002, Levy et al. 2011, Donovan et al. 2013, Powers et al. 2011). For example, Goodloe (1991) noted that mares did produce elevated titers and had suppressed follicular development for 11-13 weeks after treatment, but that all treated mares ovulated after the titer levels declined. Similarly, Elhay (2007) found that high initial titers correlated with longer-lasting ovarian and behavioral anoestrus. However, Powers et al. (2011) did not identify a threshold level of titer that was consistently indicative of suppressed reproduction despite seeing a strong correlation between antibody concentration and infertility, nor did Schulman et al. (2013) find a clear relationship between titer levels and mare acyclicity.

In many cases, young animals appear to have higher immune responses, and stronger contraceptive effects of anti-GnRH vaccines than older animals (Brown et al. 1994, Curtis et al. 2001, Stout et al. 2003, Schulman et al. 2013). Vaccinating with GonaCon at too young an age, though, may prevent effectiveness; Gionfriddo et al. (2011a) observed weak effects in 3-4 month old fawns. It has not been possible to predict which individuals of a given age class will have long-lasting immune responses to the GonaCon vaccine. Gray (2010) noted that mares in poor body condition tended to have lower contraceptive efficacy in response to GonaCon-B. Miller et al. (2013) suggested that higher parasite loads might have explained a lower immune response in free-roaming horses than had been observed in a captive trial. At this time it is unclear what the most important factors affecting efficacy are.

Several studies have monitored animal health after immunization against GnRH. GonaCon treated mares did not have any measurable difference in uterine edema (Killian 2006, 2008). Powers et al. (2011, 2013) noted no differences in blood chemistry except a mildly elevated fibrinogen level in some GonaCon treated elk. In that study, one sham-treated elk and one GonaCon treated elk each developed leukocytosis, suggesting that there may have been a causal link between the adjuvant and the effect. Curtis et al. (2008) found persistent granulomas at GonaCon-KHL injection sites three years after injection, and reduced ovary weights in treated females. Yoder and Miller (2010) found no difference in blood chemistry between GonaCon treated and control prairie dogs. One of 15 GonaCon treated cats died without explanation, and with no determination about cause of death possible based on necropsy or histology (Levy et al. 2011). Other anti-GnRH vaccine formulations have led to no detectable adverse effects (in elephants; Boedeker et al. 2011), though Imboden et al. (2006) speculated that young treated animals might conceivably have impaired hypothalamic or pituitary function.

Kirkpatrick et al. (2011) raised concerns that anti-GnRH vaccines could lead to adverse effects in other organ systems outside the reproductive system. GnRH receptors have been identified in tissues outside of the pituitary system, including in the testes and placenta (Khodr and Siler-Khodr 1980), ovary (Hsueh and Erickson 1979), bladder (Coit et al. 2009), heart (Dong et al. 2011), and central nervous system, so it is plausible that reductions in circulating GnRH levels could inhibit physiological processes in those organ systems. Kirkpatrick et al. (2011) noted elevated cardiological risks to human patients taking GnRH agonists (such as leuprolide), but the National Academy of Sciences (2013) concluded that the mechanism and results of GnRH agonists would be expected to be different from that of anti-GnRH antibodies; the former flood GnRH receptors, while the latter deprive receptors of GnRH.

Reversibility and Effects on Ovaries: PZP Vaccines

In most cases, PZP contraception appears to be temporary and reversible, with most treated mares returning to fertility over time (Kirkpatrick and Turner 2002). The ZonaStat-H formulation of the vaccine tends to confer only one year of efficacy per dose. Some studies have found that a PZP vaccine in long-lasting pellets (PZP-22) can confer multiple years of contraception (Turner et al. 2007), particularly when boostered with subsequent PZP vaccination (Rutberg et al. 2017). Other trial data, though, indicate that the pelleted vaccine may only be effective for one year (J. Turner, University of Toledo, Personal Communication to BLM).

The purpose of applying PZP vaccine treatment is to prevent mares from conceiving foals, but BLM acknowledges that long-term infertility, or permanent sterility, could be a result for some number of individual wild horses receiving PZP vaccinations. The rate of long-term or permanent sterility following vaccinations with PZP is hard to predict for individual horses, but that outcome appears to increase in likelihood as the number of doses increases (Kirkpatrick and Turner 2002). Permanent sterility for mares treated consecutively in each of 5-7 years was observed by Nuñez et al. (2010, 2017). In a graduate thesis, Knight (2014) suggested that repeated treatment with as few as three to four years of PZP treatment may lead to longer-term sterility, and that sterility may result from PZP treatment before puberty. Repeated treatment with PZP led to long-term infertility in Przewalski's horses receiving as few as one PZP booster dose (Feh 2012). However, even if some number of mares become sterile as a result of PZP treatment, that potential result would be consistent with the contraceptive purpose that motivates BLM's potential use of the vaccine.

In some number of individual mares, PZP vaccination may cause direct effects on ovaries (Gray and Cameron 2010, Joonè et al. 2017b, Joonè et al. 2017c, Joonè et al. 2017d, Nolan et al. 2018b). Joonè et al. (2017a) noted reversible effects on ovaries in mares treated with one primer dose and booster dose. Joonè et al. (2017c) and Nolan et al. (2018b) documented decreased anti-Mullerian hormone (AMH) levels in mares treated with native or recombinant PZP vaccines; AMH levels are thought to be an indicator of ovarian function. Bechert et al. (2013) found that ovarian function was affected by the SpayVac PZP vaccination, but that there were no effects on other organ systems. Mask et al. (2015) demonstrated that equine antibodies that resulted from SpayVac immunization could bind to oocytes, ZP proteins, follicular tissues, and ovarian tissues. It is possible that result is specific to the immune response to SpayVac, which may have lower PZP purity than ZonaStat or PZP-22 (Hall et al. 2016). However, in studies with native ZP proteins and recombinant ZP proteins, Joonè et al. (2017a) found transient effects on ovaries after PZP vaccination in some treated mares; normal estrus cycling had resumed 10 months after the last treatment. SpayVac is a patented formulation of PZP in liposomes that led to multiple years of infertility in some breeding trials (Killian et al. 2008, Roelle et al. 2017, Bechert and Fraker 2018), but unacceptably poor efficacy in a subsequent trial (Kane 2018). Kirkpatrick et al. (1992) noted effects on horse ovaries after three years of treatment with PZP. Observations at Assateague Island National Seashore indicated that the more times a mare is consecutively treated, the longer the time lag before fertility returns, but that even mares treated 7 consecutive years did eventually return to ovulation (Kirkpatrick and Turner 2002). Other

studies have reported that continued PZP vaccine applications may result in decreased estrogen levels (Kirkpatrick et al. 1992) but that decrease was not biologically significant, as ovulation remained similar between treated and untreated mares (Powell and Monfort 2001). Bagavant et al. (2003) demonstrated T-cell clusters on ovaries, but no loss of ovarian function after ZP protein immunization in macaques.

Reversibility and Effects on Ovaries: GnRH Vaccines
The NAS (2013) review pointed out that single doses of GonaCon-Equine do not lead to high rates of initial effectiveness, or long duration. Initial effectiveness of one dose of GonaCon-Equine vaccine appears to be lower than for a combined primer plus booster dose of the PZP vaccine Zonastat-H (Kirkpatrick et al. 2011), and the initial effect of a single GonaCon dose can be limited to as little as one breeding season. However, preliminary results on the effects of boostered doses of GonaCon-Equine indicate that it can have high efficacy and longer-lasting effects in free-roaming horses (Baker et al. 2017, 2018) than the one-year effect that is generally expected from a single booster of Zonastat-H.

Too few studies have reported on the various formulations of anti-GnRH vaccines to make generalizations about differences between products, but GonaCon formulations were consistently good at causing loss of fertility in a statistically significant fraction of treated mares for at least one year (Killian et al. 2009, Gray et al. 2010, Baker et al. 2013, 2017, 2018). With few exceptions (e.g., Goodloe 1991), anti-GnRH treated mares gave birth to fewer foals in the first season when there would be an expected contraceptive effect (Botha et al. 2008, Killian et al. 2009, Gray et al. 2010, Baker et al. 2013, 2018). Goodloe (1991) used an anti-GnRH-KHL vaccine with a triple adjuvant, in some cases attempting to deliver the vaccine to horses with a hollow-tipped ‘biobullet, ’but concluded that the vaccine was not an effective immunocontraceptive in that study.

Not all mares should be expected to respond to the GonaCon-equine vaccine; some number should be expected to continue to become pregnant and give birth to foals. In studies where mares were exposed to stallions, the fraction of treated mares that are effectively contracepted in the year after anti-GnRH vaccination varied from study to study, ranging from ~50% (Baker et al. 2017), to 61% (Gray et al. 2010), to ~90% (Killian et al. 2006, 2008, 2009). Miller et al. (2013) noted lower effectiveness in free-ranging mares (Gray et al. 2010) than captive mares (Killian et al. 2009). Some of these rates are lower than the high rate of effectiveness typically reported for the first year after PZP vaccine treatment (Kirkpatrick et al. 2011). In the one study that tested for a difference, darts and hand-injected GonaCon doses were equally effective in terms of fertility outcome (McCann et al. 2017).

In studies where mares were not exposed to stallions, the duration of effectiveness also varied. A primer and booster dose of Equity led to anoestrus for at least 3 months (Elhay et al. 2007). A primer and booster dose of Improvac also led to loss of ovarian cycling for all mares in the short term (Imboden et al. 2006, Nolan et al. 2018c). It is worth repeating that those vaccines do not have the same formulation as GonaCon.

Results from horses (Baker et al. 2017, 2018) and other species (Curtis et al. 2001) suggest that providing a booster dose of GonaCon-Equine will increase the fraction of temporarily infertile animals to higher levels than would a single vaccine dose alone.

Longer-term infertility has been observed in some mares treated with anti-GnRH vaccines, including GonaCon-Equine. In a single-dose mare captive trial with an initial year effectiveness of 94%, Killian et al. (2008) noted infertility rates of 64%, 57%, and 43% in treated mares during the following three years, while control mares in those years had infertility rates of 25%, 12%, and 0% in those years. GonaCon effectiveness in free-roaming populations was lower, with infertility rates consistently near 60% for three years after a single dose in one study (Gray et al. 2010) and annual infertility rates decreasing over time from 55% to 30% to 0% in another study with one dose (Baker et al. 2017, 2018). Similarly, gradually

increasing fertility rates were observed after single dose treatment with GonaCon in elk (Powers et al. 2011) and deer (Gionfriddo et al. 2011a).

Baker et al. (2017, 2018) observed a return to fertility over 4 years in mares treated once with GonaCon, but then noted extremely low fertility rates of 0% and 16% in the two years after the same mares were given a booster dose four years after the primer dose. Four of nine mares treated with primer and booster doses of Improvac did not return to ovulation within 2 years of the primer dose (Imboden et al. 2006), though one should probably not make conclusions about the long-term effects of GonaCon-Equine based on results from Improvac.

It is difficult to predict which females will exhibit strong or long-term immune responses to anti-GnRH vaccines (Killian et al. 2006, Miller et al. 2008, Levy et al. 2011). A number of factors may influence responses to vaccination, including age, body condition, nutrition, prior immune responses, and genetics (Cooper and Herbert 2001, Curtis et al. 2001, Powers et al. 2011). One apparent trend is that animals that are treated at a younger age, especially before puberty, may have stronger and longer-lasting responses (Brown et al. 1994, Curtis et al. 2001, Stout et al. 2003, Schulman et al. 2013). It is plausible that giving ConaGon-Equine to prepubertal mares will lead to long-lasting infertility, but that has not yet been tested.

To date, short term evaluation of anti-GnRH vaccines, show contraception appears to be temporary and reversible. Killian et al. noted long-term effects of GonaCon in some captive mares (2009). However, Baker et al. (2017) observed horses treated with GonaCon-B return to fertility after they were treated with a single primer dose; after four years, the fertility rate was indistinguishable between treated and control mares. It appears that a single dose of GonaCon results in reversible infertility. If long-term treatment resulted in permanent infertility for some treated mares, such permanent infertility fertility would be consistent with the desired effect of using GonaCon (e.g., effective contraception).

Other anti-GnRH vaccines also have had reversible effects in mares. Elhay (2007) noted a return to ovary functioning over the course of 34 weeks for 10 of 16 mares treated with Equity. That study ended at 34 weeks, so it is not clear when the other six mares would have returned to fertility. Donovan et al. (2013) found that half of mares treated with an anti-GnRH vaccine intended for dogs had returned to fertility after 40 weeks, at which point the study ended. In a study of mares treated with a primer and booster dose of Improvac, 47 of 51 treated mares had returned to ovarian cyclicity within 2 years; younger mares appeared to have longer-lasting effects than older mares (Schulman et al. 2013). Joonè et al. (2017) analyzed samples from the Schulman et al. (2013) study, and found no significant decrease in anti-Mullerian hormone (AMH) levels in mares treated with GnRH vaccine. AMH levels are thought to be an indicator of ovarian function, so results from Joonè et al. (2017) support the general view that the anoestrus resulting from GnRH vaccination is physiologically similar to typical winter anoestrus. In a small study with a non-commercial anti-GnRH vaccine (Stout et al. 2003), three of seven treated mares had returned to cyclicity within 8 weeks after delivery of the primer dose, while four others were still suppressed for 12 or more weeks. In elk, Powers et al. (2011) noted that contraception after one dose of GonaCon was reversible. In white-tailed deer, single doses of GonaCon appeared to confer two years of contraception (Miller et al. 2000). Ten of 30 domestic cows treated became pregnant within 30 weeks after the first dose of Bopriva (Balet et al. 2014).

Permanent sterility as a result of single-dose or boostered GonaCon-Equine vaccine, or other anti-GnRH vaccines, has not been recorded, but that may be because no long-term studies have tested for that effect. It is conceivable that some fraction of mares could become sterile after receiving one or more booster doses of GonaCon-Equine. If some fraction of mares treated with GonaCon-Equine were to become sterile, though, that result would be consistent with text of the WFRHBA of 1971, as amended, which allows for sterilization to achieve population goals.

In summary, based on the above results related to fertility effects of GonaCon and other anti-GnRH vaccines, application of a single dose of GonaCon-Equine to gathered or remotely-darted wild horses could be expected to prevent pregnancy in perhaps 30%-60% of mares for one year. Some smaller number of wild mares should be expected to have persistent contraception for a second year, and less still for a third year. Applying one booster dose of GonaCon to previously-treated mares may lead to four or more years with relatively high rates (80+%) of additional infertility expected (Baker et al. 2018). There is no data to support speculation regarding efficacy of multiple boosters of GonaCon-Equine; however, given it is formulated as a highly immunogenic long-lasting vaccine, it is reasonable to hypothesize that additional boosters would increase the effectiveness and duration of the vaccine.

GonaCon-Equine only affects the fertility of treated animals; untreated animals will still be expected to give birth. Even under favorable circumstances for population growth suppression, gather efficiency might not exceed 85% via helicopter, and may be less with bait and water trapping. Similarly, not all animals may be approachable for darting. The uncaptured or undarted portion of the female population would still be expected to have normally high fertility rates in any given year, though those rates could go up slightly if contraception in other mares increases forage and water availability.

Changes in hormones associated with anti-GnRH vaccination lead to measurable changes in ovarian structure and function. The volume of ovaries reduced in response to treatment (Garza et al. 1986, Dalin et al. 2002, Imboden et al. 2006, Elhay et al. 2007, Botha et al. 2008, Gionfriddo 2011a, Dalmau et al. 2015). Treatment with an anti-GnRH vaccine changes follicle development (Garza et al. 1986, Stout et al. 2003, Imboden et al. 2006, Elhay et al. 2007, Donovan et al. 2013, Powers et al. 2011, Balet et al. 2014), with the result that ovulation does not occur. A related result is that the ovaries can exhibit less activity and cycle with less regularity or not at all in anti-GnRH vaccine treated females (Goodloe 1991, Dalin et al. 2002, Imboden et al. 2006, Elhay et al. 2007, Janett et al. 2009a, Powers et al. 2011, Donovan et al. 2013). In studies where the vaccine required a booster, hormonal and associated results were generally observed within several weeks after delivery of the booster dose.

Effects on Existing Pregnancies, Foals, and Birth Phenology: PZP Vaccines
Although fetuses are not explicitly protected under the WFRHBA of 1971, as amended, it is prudent to analyze the potential effects of fertility control vaccines on developing fetuses and foals. Any impacts identified in the literature have been found to be transient, and do not influence the future reproductive capacity of offspring born to treated females.

If a mare is already pregnant, the PZP vaccine has not been shown to affect normal development of the fetus or foal, or the hormonal health of the mare with relation to pregnancy (Kirkpatrick and Turner 2003). Studies on Assateague Island (Kirkpatrick and Turner 2002) showed that once female offspring born to mares treated with PZP during pregnancy eventually breed, they produce healthy, viable foals. It is possible that there may be transitory effects on foals born to mares or jennies treated with PZP. For example, in mice, Sacco et al. (1981) found that antibodies specific to PZP can pass from mother mouse to pup via the placenta or colostrum, but that did not apparently cause any innate immune response in the offspring: the level of those antibodies were undetectable by 116 days after birth. There was no indication in that study that the fertility or ovarian function of those mouse pups was compromised, nor is BLM aware of any such results in horses or burros. Unsubstantiated, speculative connections between PZP treatment and 'foal stealing' has not been published in a peer-reviewed study and thus cannot be verified. 'Foal stealing,' where a near-term pregnant mare steals a neonate foal from a weaker mare, is unlikely to be a common behavioral result of including spayed mares in a wild horse herd. McDonnell (2012) noted that "foal stealing is rarely observed in horses, except under crowded conditions and synchronization of foaling," such as in horse feed lots. Those conditions are not likely in the wild, where pregnant mares will be widely distributed across the landscape, and where the expectation is that parturition dates would be distributed across the normal foaling season. Similarly, although Nettles (1997) noted reported stillbirths

after PZP treatments in cynomolgus monkeys, those results have not been observed in equids despite extensive use in horses and burros.

On-range observations from 20 years of application to wild horses indicate that PZP application in wild mares does not generally cause mares to give birth to foals out of season or late in the year (Kirkpatrick and Turner 2003). Nuñez's (2010) research showed that a small number of mares that had previously been treated with PZP foaled later than untreated mares and expressed the concern that this late foaling "may" impact foal survivorship and decrease band stability, or that higher levels of attention from stallions on PZP-treated mares might harm those mares. However, that paper provided no evidence that such impacts on foal survival or mare well-being actually occurred. Rubenstein (1981) called attention to a number of unique ecological features of horse herds on Atlantic barrier islands, such as where Nuñez made observations, which calls into question whether inferences drawn from island herds can be applied to western wild horse herds. Ransom et al. (2013), though, did identify a potential shift in reproductive timing as a possible drawback to prolonged treatment with PZP, stating that treated mares foaled on average 31 days later than non-treated mares. Results from Ransom et al. (2013), however, showed that over 81% of the documented births in that study were between March 1 and June 21, i.e., within the normal, peak, spring foaling season. Ransom et al. (2013) pointedly advised that managers should consider carefully before using fertility control vaccines in small refugia or rare species. Wild horses and burros managed by BLM do not generally occur in isolated refugia, nor are they at all rare species. The US Fish and Wildlife Service denied a petition to list wild horses as endangered (USFWS 2015). Moreover, any effect of shifting birth phenology was not observed uniformly: in two of three PZP-treated wild horse populations studied by Ransom et al. (2013), foaling season of treated mares extended three weeks and 3.5 months, respectively, beyond that of untreated mares. In the other population, the treated mares foaled within the same time period as the untreated mares. Furthermore, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season. If there are shifts in birth phenology, though, it is reasonable to assume that some negative effects on foal survival for a small number of foals might result from particularly severe weather events (Nuñez et al. 2018).

Effects on Existing Pregnancies, Foals, and Birth Phenology: GnRH Vaccines
Although fetuses are not explicitly protected under the WFRHBA of 1971, as amended, it is prudent to analyze the potential effects of fertility control vaccines on developing fetuses and foals. Any impacts identified in the literature have been found to be transient, and do not influence the future reproductive capacity of offspring born to treated females.

GonaCon and other anti-GnRH vaccines can be injected while a female is pregnant (Miller et al. 2000, Powers et al. 2011, Baker et al. 2013) – in such a case, a successfully contracepted mare will be expected to give birth during the following foaling season, but to be infertile during the same year's breeding season. Thus, a mare injected in November of 2018 would not show the contraceptive effect (i.e., no new foal) until spring of 2020.

GonaCon had no apparent effect on pregnancies in progress, foaling success, or the health of offspring, in horses that were immunized in October (Baker et al. 2013), elk immunized 80-100 days into gestation (Powers et al. 2011, 2013), or deer immunized in February (Miller et al. 2000). Kirkpatrick et al. (2011) noted that anti-GnRH immunization is not expected to cause hormonal changes that would lead to abortion in the horse, but this may not be true for the first 6 weeks of pregnancy (NAS 2013). Curtis et al. (2011) noted that GonaCon-KHL treated white tailed deer had lower twinning rates than controls, but speculated that the difference could be due to poorer sperm quality late in the breeding season, when the treated does did become pregnant. Goodloe (1991) found no difference in foal production between treated and control animals.

Offspring of anti-GnRH vaccine treated mothers could exhibit an immune response to GnRH (Khodr and

Siler-Khodr 1980), as antibodies from the mother could pass to the offspring through the placenta or colostrum. In the most extensive study of long-term effects of GonaCon immunization on offspring, Powers et al. (2012) monitored 15 elk fawns born to GonaCon treated cows. Of those, 5 had low titers at birth and 10 had high titer levels at birth. All 15 were of normal weight at birth, and developed normal endocrine profiles, hypothalamic GnRH content, pituitary gonadotropin content, gonad structure, and gametogenesis. All the females became pregnant in their second reproductive season, as is typical. All males showed normal development of secondary sexual characteristics. Powers et al. (2012) concluded that suppressing GnRH in the neonatal period did not alter long-term reproductive function in either male or female offspring. Miller et al. (2013) report elevated anti-GnRH antibody titers in fawns born to treated white tailed deer, but those dropped to normal levels in 11 of 12 of those fawns, which came into breeding condition; the remaining fawn was infertile for three years.

Direct effects on foal survival are equivocal in the literature. Goodloe (1991), reported lower foal survival for a small sample of foals born to anti-GnRH treated mares, but she did not assess other possible explanatory factors such as mare social status, age, body condition, or habitat in her analysis (NAS 2013). Gray et al. (2010) found no difference in foal survival in foals born to free-roaming mares treated with GonaCon.

There is little empirical information available to evaluate the effects of GnRH vaccination on foaling phenology, but those effects are likely to be similar to those for PZP vaccine treated mares in which the effects of the vaccine wear off. It is possible that immunocontracepted mares returning to fertility late in the breeding season could give birth to foals at a time that is out of the normal range (Nuñez et al. 2010, Ransom et al 2013). Curtis et al. (2001) did observe a slightly later fawning date for GonaCon treated deer in the second year after treatment, when some does regained fertility late in the breeding season. In anti-GnRH vaccine trials in free-roaming horses, there were no published differences in mean date of foal production (Goodloe 1991, Gray et al. 2010). Unpublished results from an ongoing study of GonaCon treated free-roaming mares indicate that some degree of seasonal foaling is possible (D. Baker, Colorado State University, personal communication to Paul Griffin, BLM WH&B Research Coordinator). Because of the concern that contraception could lead to shifts in the timing of parturitions for some treated animals, Ransom et al. (2013) advised that managers should consider carefully before using PZP immunocontraception in small refugia or rare species; the same considerations could be advised for use of GonaCon, but wild horses and burros in most areas do not generally occur in isolated refugia, they are not a rare species at the regional, national, or international level, and genetically they represent descendants of domestic livestock with most populations containing few if any unique alleles (NAS 2013). Moreover, in PZP-treated horses that did have some degree of parturition date shift, Ransom et al. (2013) found no negative impacts on foal survival even with an extended birthing season; however, this may be more related to stochastic, inclement weather events than extended foaling seasons. If there were to be a shift in foaling date for some treated mares, the effect on foal survival may depend on weather severity and local conditions; for example, Ransom et al. (2013) did not find consistent effects across study sites.

Effects of Marking and Injection

Standard practices require that immunocontraceptive-treated animals be readily identifiable, either via brand marks or unique coloration (BLM 2010). Some level of transient stress is likely to result in newly captured mares that do not have markings associated with previous fertility control treatments. It is difficult to compare that level of temporary stress with the long-term stress that can result from food and water limitation on the range (e.g., Creel et al. 2013). Handling may include freeze-marking, for the purpose of identifying that mare and identifying her vaccine treatment history. Under past management practices, captured mares experienced increased stress levels from handling (Ashley and Holcombe 2001), but BLM has instituted guidelines to reduce the sources of handling stress in captured animals (BLM 2015).

Most mares recover from the stress of capture and handling quickly once released back to the range, and none are expected to suffer serious long term effects from the fertility control injections, other than the direct consequence of becoming temporarily infertile. Injection site reactions associated with fertility control treatments are possible in treated mares (Roelle and Ransom 2009, Bechert et al. 2013, French et al. 2017, Baker et al. 2018), but swelling or local reactions at the injection site are expected to be minor in nature. Roelle and Ransom (2009) found that the most time-efficient method for applying PZP is by hand-delivered injection of 2-year pellets when horses are gathered. They observed only two instances of swelling from that technique. Whether injection is by hand or via darting, GonaCon-Equine is associated with some degree of inflammation, swelling, and the potential for abscesses at the injection site (Baker et al. 2013). Swelling or local reactions at the injection site are generally expected to be minor in nature, but some may develop into draining abscesses. Use of remotely delivered vaccine is generally limited to populations where individual animals can be accurately identified and repeatedly approached. The dart-delivered PZP formulation produced injection-site reactions of varying intensity, though none of the observed reactions appeared debilitating to the animals (Roelle and Ransom 2009) but that was not observed with dart-delivered GonaCon (McCann et al. 2017). Joonè et al. (2017a) found that injection site reactions had healed in most mares within 3 months after the booster dose, and that they did not affect movement or cause fever.

Long-lasting nodules observed did not appear to change any animal's range of movement or locomotor patterns and in most cases did not appear to differ in magnitude from naturally occurring injuries or scars. Mares treated with one formulation of GnRH-KHL vaccine developed pyogenic abscesses (Goodloe 1991). Miller et al. (2008) noted that the water and oil emulsion in GonaCon will often cause cysts, granulomas, or sterile abscesses at injection sites; in some cases, a sterile abscess may develop into a draining abscess. In elk treated with GonaCon, Powers et al. (2011) noted up to 35% of treated elk had an abscess form, despite the injection sites first being clipped and swabbed with alcohol. Even in studies where swelling and visible abscesses followed GonaCon immunization, the longer term nodules observed did not appear to change any animal's range of movement or locomotor patterns (Powers et al. 2013, Baker et al. 2017, 2018). The result that other formulations of anti-GnRH vaccine may be associated with less notable injection site reactions in horses may indicate that the adjuvant formulation in GonaCon leads a single dose to cause a stronger immune reaction than the adjuvants used in other anti-GnRH vaccines. Despite that, a booster dose of GonaCon-Equine appears to be more effective than a primer dose alone (Baker et al. 2017). Horses injected in the hip with Improvac showed only transient reactions that disappeared within 6 days in one study (Botha et al. 2008), but stiffness and swelling that lasted 5 days were noted in another study where horses received Improvac in the neck (Imboden et al. 2006). In an examination of the GnRH vaccine Equity™, IM injections in the neck led to transient reactions that resolved within a week in some treated animals (Elhay et al. 2007). Donovan et al. noted no reactions to the canine anti-GnRH vaccine (2013). In cows treated with Bopriva there was a mildly elevated body temperature and mild swelling at injection sites that subsided within 2 weeks (Balet et al. 2014).

Indirect Effects: PZP Vaccines

One expected long-term, indirect effect on wild horses treated with fertility control would be an improvement in their overall health (Turner and Kirkpatrick 2002). Many treated mares would not experience the biological stress of reproduction, foaling and lactation as frequently as untreated mares. The observable measure of improved health is higher body condition scores (Nuñez et al. 2010). After a treated mare returns to fertility, her future foals would be expected to be healthier overall, and would benefit from improved nutritional quality in the mare's milk. This is particularly to be expected if there is an improvement in rangeland forage quality at the same time, due to reduced wild horse population size. Past application of fertility control has shown that mares' overall health and body condition remains improved even after fertility resumes. PZP treatment may increase mare survival rates, leading to longer

potential lifespan (Turner and Kirkpatrick 2002, Ransom et al. 2014a) that may be as much as 5-10 years (NPS 2008). To the extent that this happens, changes in lifespan and decreased foaling rates could combine to cause changes in overall age structure in a treated herd (i.e., Turner and Kirkpatrick 2002, Roelle et al. 2010), with a greater prevalence of older mares in the herd (Gross 2000, NPS 2008). Observations of mares treated in past gathers showed that many of the treated mares were larger than, maintained higher body condition than, and had larger healthy foals than untreated mares (BLM, anecdotal observations).

Following resumption of fertility, the proportion of mares that conceive and foal could be increased due to their increased fitness; this has been called a 'rebound effect.' Elevated fertility rates have been observed after horse gathers and removals (Kirkpatrick and Turner 1991). If repeated contraceptive treatment leads to a prolonged contraceptive effect, then that may minimize or delay the hypothesized rebound effect. Selectively applying contraception to older animals and returning them to the range could reduce long-term holding costs for such horses, which are difficult to adopt, and may reduce the compensatory reproduction that often follows removals (Kirkpatrick and Turner 1991).

Because successful fertility control in a given herd reduces foaling rates and population growth rates, another indirect effect should be to reduce the number of wild horses that have to be removed over time to achieve and maintain the established AML. Contraception may change a herd's age structure, with a relative increase in the fraction of older animals in the herd (NPS 2008). Reducing the numbers of wild horses that would have to be removed in future gathers could allow for removal of younger, more easily adoptable excess wild horses, and thereby could eliminate the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding.

A principal motivation for use of contraceptive vaccines or sex ratio manipulation is to reduce population growth rates and maintain herd sizes at AML. Where successful, this should allow for continued and increased environmental improvements to range conditions within the project area, which would have long-term benefits to wild horse and burro habitat quality, and well-being of animals living on the range. As the population nears or is maintained at the level necessary to achieve a thriving natural ecological balance, vegetation resources would be expected to recover, improving the forage available. With rangeland conditions more closely approaching a thriving natural ecological balance, and with a less concentrated distribution of wild horses and burros, there should also be less trailing and concentrated use of water sources. Lower population density should lead to reduced competition among wild horses using the water sources, and less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Among mares in the herd that remain fertile, a higher level of physical health and future reproductive success would be expected in areas where lower horse and burro population sizes lead to increases in water and forage resources. While it is conceivable that widespread and continued treatment with fertility control vaccines could reduce the birth rates of the population to such a point that birth is consistently below mortality, that outcome is not likely unless a very high fraction of the mares present are all treated in almost every year.

Indirect Effects: GnRH Vaccines

As noted above to PZP vaccines, an expected long-term, indirect effect on wild horses treated with fertility control would be an improvement in their overall health. Body condition of anti-GnRH-treated females was equal to or better than that of control females in published studies. Ransom et al. (2014b) observed no difference in mean body condition between GonaCon-B treated mares and controls. Goodloe (1991) found that GnRH-KHL treated mares had higher survival rates than untreated controls. In other species, treated deer had better body condition than controls (Gionfriddo et al. 2011b), treated cats gained more weight than controls (Levy et al. 2011), as did treated young female pigs (Bohrer et al. 2014).

Following resumption of fertility, the proportion of mares that conceive and foal could be increased due to their increased fitness; this has been called by some a 'rebound effect.' Elevated fertility rates have been observed after horse gathers and removals (Kirkpatrick and Turner 1991). If repeated contraceptive treatment leads to a prolonged contraceptive effect, then that may minimize or delay the hypothesized rebound effect. Selectively applying contraception to older animals and returning them to the range could reduce long-term holding costs for such horses, which are difficult to adopt, and could negate the compensatory reproduction that can follow removals (Kirkpatrick and Turner 1991).

Because successful fertility control would reduce foaling rates and population growth rates, another indirect effect would be to reduce the number of wild horses that have to be removed over time to achieve and maintain the established AML. Contraception would be expected to lead to a relative increase in the fraction of older animals in the herd. Reducing the numbers of wild horses that would have to be removed in future gathers could allow for removal of younger, more easily adoptable excess wild horses, and thereby could eliminate the need to send additional excess horses from this area to off-range holding corrals or pastures for long-term holding. Among mares in the herd that remain fertile, a high level of physical health and future reproductive success would be expected because reduced population sizes should lead to more availability of water and forage resources per capita.

Reduced population growth rates and smaller population sizes could also allow for continued and increased environmental improvements to range conditions within the project area, which would have long-term benefits to wild horse habitat quality. As the local horse abundance nears or is maintained at the level necessary to achieve a thriving natural ecological balance, vegetation resources would be expected to recover, improving the forage available to wild horses and wildlife throughout the area. With rangeland conditions more closely approaching a thriving natural ecological balance, and with a less concentrated distribution of wild horses across the range, there should also be less trailing and concentrated use of water sources. Lower population density would be expected to lead to reduced competition among wild horses using the water sources, and less fighting among horses accessing water sources. Water quality and quantity would continue to improve to the benefit of all rangeland users including wild horses. Wild horses would also have to travel less distance back and forth between water and desirable foraging areas. Should GonaCon-Equine treatment, including booster doses, continue into the future, with treatments given on a schedule to maintain a lowered level of fertility in the herd, the chronic cycle of overpopulation and large gathers and removals might no longer occur, but instead a consistent abundance of wild horses could be maintained, resulting in continued improvement of overall habitat conditions and animal health. While it is conceivable that widespread and continued treatment with GonaCon-Equine could reduce the birth rates of the population to such a point that birth is consistently below mortality, that outcome is not likely unless a very high fraction of the mares present are all treated with primer and booster doses, and perhaps repeated booster doses.

Behavioral Effects: PZP Vaccines

Behavioral difference, compared to mares that are fertile, should be considered as potential results of successful contraception. The NAS report (2013) noted that all forms of fertility suppression have effects on mare behavior, mostly because of the lack of pregnancy and foaling, and concluded that fertility control vaccines were among the most promising fertility control methods for wild horses and burros. The resulting impacts may be seen as neutral in the sense that a wide range of natural behaviors is already observable in untreated wild horses, or mildly adverse in the sense that effects are expected to be transient and to not affect all treated animals.

PZP vaccine-treated mares may continue estrus cycles throughout the breeding season. Ransom and Cade (2009) delineated wild horse behaviors. Ransom et al. (2010) found no differences in how PZP-treated and untreated mares allocated their time between feeding, resting, travel, maintenance, and most social behaviors in three populations of wild horses, which is consistent with Powell's (1999) findings in

another population. Likewise, body condition of PZP-treated and control mares did not differ between treatment groups in Ransom et al.'s (2010) study. Nuñez (2010) found that PZP-treated mares had higher body condition than control mares in another population, presumably because energy expenditure was reduced by the absence of pregnancy and lactation. Knight (2014) found that PZP-treated mares had better body condition, lived longer and switched harems more frequently, while mares that foaled spent more time concentrating on grazing and lactation and had lower overall body condition.

In two studies involving a total of four wild horse populations, both Nuñez et al. (2009) and Ransom et al. (2010) found that PZP vaccine treated mares were involved in reproductive interactions with stallions more often than control mares, which is not surprising given the evidence that PZP-treated females of other mammal species can regularly demonstrate estrus behavior while contracepted (Shumake and Killian 1997, Heilmann et al. 1998, Curtis et al. 2001, Duncan et al. 2017). There was no evidence, though, that mare welfare was affected by the increased level of herding by stallions noted in Ransom et al. (2010). Nuñez's later analysis (2017) noted no difference in mare reproductive behavior as a function of contraception history.

Ransom et al. (2010) found that control mares were herded by stallions more frequently than PZP-treated mares, and Nuñez et al. (2009, 2014, 2017, 2018) found that PZP-treated mares exhibited higher infidelity to their band stallion during the non-breeding season than control mares. Madosky et al. (2010) and Knight (2014) found this infidelity was also evident during the breeding season in the same population that Nuñez et al. (2009, 2010, 2014, 2017, 2018) studied. Nuñez et al. (2014, 2017, 2018) concluded that PZP-treated mares changing bands more frequently than control mares could lead to band instability. Nuñez et al. (2009), though, cautioned against generalizing from that island population to other herds. Also, despite any potential changes in band infidelity due to PZP vaccination, horses continued to live in social groups with dominant stallions and one or more mares. Nuñez et al. (2014) found elevated levels of fecal cortisol, a marker of physiological stress, in mares that changed bands. The research is inconclusive as to whether all the mares' movements between bands were related to the PZP treatments themselves or the fact that the mares were not nursing a foal, and did not demonstrate any long-term negative consequence of the transiently elevated cortisol levels. In separate work in a long-term study of semi-feral Konik ponies, Jaworska et al. (2020) showed that neither infanticide nor feticide resulted for mares and their foals after a change in dominant stallion. Nuñez et al. 2014 wrote that these effects "…may be of limited concern when population reduction is an urgent priority." Nuñez (2018) and Jones et al. (2019, 2020) noted that band stallions of mares that have received PZP treatment can exhibit changes in behavior and physiology. Nuñez (2018) cautioned that PZP use may limit the ability of mares to return to fertility, but also noted that, "such aggressive treatments may be necessary when rapid reductions in animal numbers are of paramount importance…If the primary management goal is to reduce population size, it is unlikely (and perhaps less important) that managers achieve a balance between population control and the maintenance of more typical feral horse behavior and physiology."

In contrast to transient stresses, Creel et al. (2013) highlight that variation in population density is one of the most well-established causal factors of chronic activation of the hypothalamic-pituitary-adrenal axis, which mediates stress hormones; high population densities and competition for resources can cause chronic stress. Creel et al. (2013) also state that "…there is little consistent evidence for a negative association between elevated baseline glucocorticoids and fitness." Band fidelity is not an aspect of wild horse biology that is specifically protected by the WFRHBA of 1971. It is also notable that Ransom et al. (2014b) found higher group fidelity after a herd had been gathered and treated with a contraceptive vaccine; in that case, the researchers postulated that higher fidelity may have been facilitated by the decreased competition for forage after excess horses were removed. At the population level, available research does not provide evidence of the loss of harem structure among any herds treated with PZP. No biologically significant negative impacts on the overall animals or populations overall, long-term welfare or well-being have been established in these studies.

The National Research Council (2013) found that harem changing was not likely to result in serious adverse effects for treated mares:

> "The studies on Shackleford Banks (Nuñez et al., 2009; Madosky et al., 2010) suggest that there is an interaction between pregnancy and social cohesion. The importance of harem stability to mare well-being is not clear, but considering the relatively large number of free-ranging mares that have been treated with liquid PZP in a variety of ecological settings, the likelihood of serious adverse effects seem low."

Nuñez (2010) stated that not all populations will respond similarly to PZP treatment. Differences in habitat, resource availability, and demography among conspecific populations will undoubtedly affect their physiological and behavioral responses to PZP contraception, and may be considered. Kirkpatrick et al. (2010) concluded that: "the larger question is, even if subtle alterations in behavior may occur, this is still far better than the alternative," and that the "…other victory for horses is that every mare prevented from being removed, by virtue of contraception, is a mare that will only be delaying her reproduction rather than being eliminated permanently from the range. This preserves herd genetics, while gathers and adoption do not."

The NAS report (2013) provides a comprehensive review of the literature on the behavioral effects of contraception that puts research up to that date by Nuñez et al. (2009, 2010) into the broader context of all of the available scientific literature, and cautions, based on its extensive review of the literature that:

> ". . . in no case can the committee conclude from the published research that the behavior differences observed are due to a particular compound rather than to the fact that treated animals had no offspring during the study. That must be borne in mind particularly in interpreting long-term impacts of contraception (e.g., repeated years of reproductive "failure" due to contraception)."

Behavioral Effects: GnRH Vaccines

The result that GonaCon treated mares may have suppressed estrous cycles throughout the breeding season can lead treated mares to behave in ways that are functionally similar to pregnant mares. Where it is successful in mares, GonaCon and other anti-GnRH vaccines are expected to induce fewer estrous cycles when compared to non-pregnant control mares. This has been observed in many studies (Garza et al. 1986, Curtis et al. 2001, Dalin et al. 2002, Killian et al. 2006, Dalmau et al. 2015). Females treated with GonaCon had fewer estrous cycles than control or PZP-treated mares (Killian et al. 2006) or deer (Curtis et al. 2001). Thus, any concerns about PZP treated mares receiving more courting and breeding behaviors from stallions (Nuñez et al. 2009, Ransom et al. 2010) are not generally expected to be a concern for mares treated with anti-GnRH vaccines (Botha et al. 2008).

Ransom et al. (2014b) and Baker et al. (2018) found that GonaCon treated mares had similar rates of reproductive behaviors that were similar to those of pregnant mares. Among other potential causes, the reduction in progesterone levels in treated females may lead to a reduction in behaviors associated with reproduction. Despite this, some females treated with GonaCon or other anti-GnRH vaccines did continue to exhibit reproductive behaviors, albeit at irregular intervals and durations (Dalin et al. 2002, Stout et al. 2003, Imboden et al. 2006), which is a result that is similar to spayed (ovariectomized) mares (Asa et al. 1980). Gray et al. (2009a) and Baker et al. (2018) found no difference in sexual behaviors in mares treated with GonaCon and untreated mares. When progesterone levels are low, small changes in estradiol concentration can foster reproductive estrous behaviors (Imboden et al. 2006). Owners of anti-GnRH vaccine treated mares reported a reduced number of estrous-related behaviors under saddle (Donovan et al. 2013). Treated mares may refrain from reproductive behavior even after ovaries return to cyclicity (Elhay et al. 2007). Studies in elk found that GonaCon treated cows had equal levels of precopulatory behaviors as controls (Powers et al. 2011), though bull elk paid more attention to treated cows late in the

breeding season, after control cows were already pregnant (Powers et al. 2011).

Stallion herding of mares, and harem switching by mares are two behaviors related to reproduction that might change as a result of contraception. Ransom et al. (2014b) observed a 50% decrease in herding behavior by stallions after the free-roaming horse population at Theodore Roosevelt National Park was reduced via a gather, and mares there were treated with GonaCon-B. The increased harem tending behaviors by stallions were directed to both treated and control mores. It is difficult to separate any effect of GonaCon in this study from changes in horse density and forage following horse removals.

With respect to treatment with GonaCon or other anti-GnRH vaccines, it is probably less likely that treated mares will switch harems at higher rates than untreated animals, because treated mares are similar to pregnant mares in their behaviors (Ransom et al. 2014b). Indeed, Gray et al. (2009a) found no difference in band fidelity in a free-roaming population of horses with GonaCon treated mares, despite differences in foal production between treated and untreated mares. Ransom et al. (2014b) actually found increased levels of band fidelity after treatment, though this may have been partially a result of changes in overall horse density and forage availability.

Gray et al. (2009) and Ransom et al. (2014b) monitored non-reproductive behaviors in GonaCon treated populations of free-roaming horses. Gray et al. (2009a) found no difference between treated and untreated mares in terms of activity budget, sexual behavior, proximity of mares to stallions, or aggression. Ransom et al. (2014b) found only minimal differences between treated and untreated mare time budgets, but those differences were consistent with differences in the metabolic demands of pregnancy and lactation in untreated mares, as opposed to non-pregnant treated mares.

Genetic Effects of Fertility Control Vaccines

In HMAs where large numbers of wild horses have recent and / or an ongoing influx of breeding animals from other areas with wild or feral horses, contraception is not expected to cause an unacceptable loss of genetic diversity or an unacceptable increase in the inbreeding coefficient. In any diploid population, the loss of genetic diversity through inbreeding or drift can be prevented by large effective breeding population sizes (Wright 1931) or by introducing new potential breeding animals (Mills and Allendorf 1996). The NAS report (2013) recommended that single HMAs should not be considered as isolated genetic populations. Rather, managed herds of wild horses should be considered as components of interacting metapopulations, with the potential for interchange of individuals and genes taking place as a result of both natural and human-facilitated movements. Introducing 1-2 mares every generation (about every 10 years) is a standard management technique that can alleviated potential inbreeding concerns (BLM 2010).

In the last 10 years, there has been a high realized growth rate of wild horses in most areas administered by the BLM, such that most alleles that are present in any given mare are likely to already be well represented in her siblings, cousins, and more distant relatives. With the exception of horses in a small number of well-known HMAs that contain a relatively high fraction of alleles associated with old Spanish horse breeds (NAS 2013), the genetic composition of wild horses in lands administered by the BLM is consistent with admixtures from domestic breeds. As a result, in most HMAs, applying fertility control to a subset of mares is not expected to cause irreparable loss of genetic diversity. Improved longevity and an aging population are expected results of contraceptive treatment that can provide for lengthening generation time; this result would be expected to slow the rate of genetic diversity loss (Hailer et al. 2006). Based on a population model, Gross (2000) found that a strategy to preferentially treat young animals with a contraceptive led to more genetic diversity being retained than either a strategy that preferentially treats older animals, or a strategy with periodic gathers and removals.

Even if it is the case that repeated treatment with a fertility control vaccine may lead to prolonged

infertility, or even sterility in some mares, most HMAs have only a low risk of loss of genetic diversity if logistically realistic rates of contraception are applied to mares. Wild horses in most herd management areas are descendants of a diverse range of ancestors coming from many breeds of domestic horses. As such, the existing genetic diversity in the majority of HMAs does not contain unique or historically unusual genetic markers. Past interchange between HMAs, either through natural dispersal or through assisted migration (i.e., human movement of horses) means that many HMAs are effectively indistinguishable and interchangeable in terms of their genetic composition (i.e., see the table of Fst vales *in* NAS 2013). Roelle and Oyler-McCance (2015) used the VORTEX population model to simulate how different rates of mare sterility would influence population persistence and genetic diversity, in populations with high or low starting levels of genetic diversity, various starting population sizes, and various annual population growth rates. Their results show that the risk of the loss of genetic heterozygosity is extremely low except in case where all of the following conditions are met: starting levels of genetic diversity are low, initial population size is 100 or less, the intrinsic population growth rate is low (5% per year), and very large fractions of the female population are permanently sterilized.

It is worth noting that, although maintenance of genetic diversity at the scale of the overall population of wild horses is an intuitive management goal, there are no existing laws or policies that require BLM to maintain genetic diversity at the scale of the individual herd management area or complex. Also, there is no Bureau-wide policy that requires BLM to allow each female in a herd to reproduce before she is treated with contraceptives.

One concern that has been raised with regards to genetic diversity is that treatment with immunocontraceptives could possibly lead to an evolutionary increase in the frequency of individuals whose genetic composition fosters weak immune responses (Cooper and Larson 2006, Ransom et al. 2014a).Many factors influence the strength of a vaccinated individual's immune response, potentially including genetics, but also nutrition, body condition, and prior immune responses to pathogens or other antigens (Powers et al. 2013). This premise is based on an assumption that lack of response to any given fertility control vaccine is a heritable trait, and that the frequency of that trait will increase over time in a population of vaccine-treated animals. Cooper and Herbert (2001) reviewed the topic, in the context of concerns about the long-term effectiveness of immunocontraceptives as a control agent for exotic species in Australia. They argue that imunocontraception could be a strong selective pressure, and that selecting for reproduction in individuals with poor immune response could lead to a general decline in immune function in populations where such evolution takes place. Other authors have also speculated that differences in antibody titer responses could be partially due to genetic differences between animals (Curtis et al. 2001, Herbert and Trigg 2005). However, Magiafolou et al. (2013) clarify that if the variation in immune response is due to environmental factors (i.e., body condition, social rank) and not due to genetic factors, then there will be no expected effect of the immune phenotype on future generations. It is possible that general health, as measured by body condition, can have a causal role in determining immune response, with animals in poor condition demonstrating poor immune reactions (NAS 2013).

Correlations between physical factors and immune response would not preclude, though, that there could also be a heritable response to immunocontraception. In studies not directly related to immunocontraception, immune response has been shown to be heritable (Kean et al. 1994, Sarker et al. 1999). Unfortunately, predictions about the long-term, population-level evolutionary response to immunocontraceptive treatments are speculative at this point, with results likely to depend on several factors, including: the strength of the genetic predisposition to not respond to the fertility control vaccine; the heritability of that gene or genes; the initial prevalence of that gene or genes; the number of mares treated with a primer dose of the vaccine (which generally has a short-acting effect); the number of mares treated with one or more booster doses of the vaccine; and the actual size of the genetically-interacting metapopulation of horses within which the vaccine treatment takes place.

BLM is not aware of any studies that have quantified the heritability of a lack of response to immunocontraception such as PZP vaccine or GonaCon-Equine in horses or burros. At this point, there are no studies available from which one could make conclusions about the long-term effects of sustained and widespread immunocontraception treatments on population-wide immune function. Although a few, generally isolated, feral horse populations have been treated with high fractions of mares receiving PZP immunocontraception for long-term population control (e.g., Assateague Island National Park, and Pryor Mountains Herd Management Area), no studies have tested for changes in immune competence in those areas. Relative to the large number of free-roaming feral horses in the western United States, immunocontraception has not been, and is not expected to be used in the type of widespread or prolonged manner that might be required to cause a detectable evolutionary response.

The Little Fish Lake JMA would have only a low risk of loss of genetic diversity if logistically realistic rates of PZP vaccine contraception are applied to mares. After the initial gather, subsequent PZP vaccine and/or would take place only after gathers, but also could take place through remote field darting. Wild horses in most HMAs are descendants of a diverse range of ancestors coming from many breeds of domestic horses, and this is apparently true in the Little Fish Lake JMA as well. The genetic diversity of the Little Fish Lake herd was sampled in 2005, 2008, and 2015. Sampling showed an increase in genetic diversity, as measured by observed heterozygosity, from being low and genetically stable in 2005 and 2008 to being higher than the average observed for feral horses in 2015. The Little Fish Lake herd genetic samples in each case led to results that indicated "…a herd with mixed origins". The results of Little Fish Lake herd sampling since 2005 range from similarity to New World Spanish breeds with "strong Quarter horse (or less likely thoroughbred) influence" (Cothran 2005, 2008), to similarity to light racing and riding breeds, followed closely by the Oriental and Arabian breeds (Cothran 2015). Genetic monitoring identified a moderate proportion of rare alleles in 2015 only, and while "genetic variability of this herd in general is on the high side with only a moderate percentage of variation that is at risk", continued genetic monitoring of the herd was recommended (Cothran 2015). Genetic diversity monitoring indicates that horses from Little Fish Lake had higher than average observed heterozygosity in all three sampling events, despite a number of past gathers having taken place, and identified no unique alleles in Little Fish Lake HMA (Cothran 2010, 2015, 2017). Pairwise Fst values show that horses from Little Fish Lake are extremely similar, genetically, to many other sampled wild horse herds (NRC 2013, Appendix F). More specifically, the samples from Little Fish Lake had pairwise Fst values that were lower than 0.05, when compared with over 60 other sample sets. For reference, values of pairwise Fst under approximately 0.05 are taken to e, values of 0.10 indicate very little differentiation, and only if values are above about 0.15 are any two sampled subpopulations considered to have evidence of elevated differentiation (Frankham et al. 2010). Cothran (2015) found that sampled horses from Little Fish Lake HMA clustered closely with animals from the Seven Mile herd which is adjacent to Little Fish Lake. This historic, and probably ongoing, interchange would be expected to have the effect of maintaining relatively high levels of genetic diversity., even despite the periodic removals that have taken place in the Little Fish Lake HMA and having some number of infertile animals in the herd

After the 2017 gather, 68 samples were analyzed for ancestral data. Once compiled, the data showed that the highest breed similarities were Hackney Horse, Quarter Horse, Shetland Pony and Garanno. The Shetland Pony is likely indicative of draft breeds. An HMA ancestral comparison was also completed comparing the Little Fish Lake HMA samples to the North and South Stone Cabin and Saulsbury HMAs and Nevada Wild Horse Range as well as to itself. The highest results (besides the Little Fish Lake HMA) were for the Nevada Wild Horse Range, followed by South Stone Cabin which indicates a common ancestry and likely continuous wild horse movement between these areas.

The Little Fish Lake JMA is contiguous with the Seven Mile HMA to the north, Dobbin Summit and Monitor WHTs to the west, and the Stone Cabin WHT to the south. Some movement between HMAs and

WHTs is expected, particularly between the Seven Mile and Little Fish Lake HMAs due to the terrain, discontinuous fencing, and known trailing and horse movement patterns. Movement has been documented between the Little Fish Lake HMA and the Nevada Wild Horse Range. Refer to the Supplemental Information document for an overview map of nearby HMAs. Prior to 2009, the U.S. Highway 6 right-of-way was not fenced, allowing wild horse movement to occur between the Little Fish Lake, and Hot Creek HMAs. Though the degree of movement is unknown, adequate interchange between HMAs within this "metapopulation" likely occurs to maintain the genetic diversity of the Little Fish Lake JMA, which is supported by the results of past genetic analysis. Refer to Map 2 in Section 12 of this document for an overview of nearby HMAs.

Roelle and Oyler-McCance (2015) used the VORTEX population model to simulate how different rates of mare sterility would influence population persistence and genetic diversity, in populations with high or low starting levels of genetic diversity, various starting population sizes, and various annual population growth rates. Their results show that the risk of the loss of genetic heterozygosity is extremely low except in case where all of the following conditions are met: starting levels of genetic diversity are low, initial population size is 100 or less, the intrinsic population growth rate is low (5% per year), and very large fractions of the female population are permanently sterilized.

8.2 Effects of Intrauterine Devices (IUDs)

Based on promising results from published, peer-reviewed studies in domestic mares, BLM has begun to use IUDs to control fertility as a wild horse and burro fertility control method on the range. The initial management use was in mares from the Swasey HMA, in Utah. The BLM has supported and continues to support research into the development and testing of effective and safe IUDs for use in wild horse mares (Baldrighi et al. 2017, Holyoak et al. 2021). However, existing literature on the use of IUDs in horses allows for inferences about expected effects of any management alternatives that might include use of IUDs, and support the apparent safety and efficacy of some types of IUDs for use in horses. Overall, as with other methods of population growth suppression, use of IUDs and other fertility control measures are expected to help reduce population growth rates, extend the time interval between gathers, and reduce the total number of excess animals that will need to be removed from the range.

The 2013 National Academies of Sciences (NAS) report considered IUDs, and suggested that research should test whether IUDs cause uterine inflammation, and should also test how well IUDs stay in mares that live and breed with fertile stallions. Since that report, a recent study by Holyoak et al. (2021) indicate that a flexible, inert, y-shaped, medical-grade silicone IUD design prevented pregnancies in all the domestic mares that retained the device, even when exposed to fertile stallions. Domestic mares in that study lived in large pastures, mating with fertile stallions. Biweekly ultrasound examinations showed that IUDs stayed in 75% of treated mares over the course of two breeding seasons. The IUDs were then removed so the researchers could monitor the mares' return to fertility. In that study, uterine health, as measured in terms of inflammation, was not seriously affected by the IUDs, and most mares became pregnant within months after IUD removal. The overall results are consistent with results from an earlier study (Daels and Hughes 1995), which used O-shaped silicone IUDs. Similarly, a flexible IUD with three components connected by magnetic force (the 'iUPOD') was retained over 90 days in mares living and breeding with a fertile stallion; after IUD removal, the majority of mares became pregnant in the following breeding season (Hoopes et al. 2021).

IUDs are considered a temporary fertility control method that does not generally cause future sterility (Daels and Hughes 1995). Use of IUDs is an effective fertility control method in women, and IUDs have historically been used in livestock management, including in domestic horses. Insertion of an IUD can be a very rapid procedure, but it does require the mare to be temporarily restrained, such as in a squeeze

chute. IUDs in mares may cause physiological effects including discomfort, infection, perforation of the uterus if the IUD is hard and angular, endometritis, uterine edema (Killian et al. 2008), and pyometra (Klabnik-Bradford et al. 2013). In women, deaths attributable to IUD use may be as low as 1.06 per million (Daels and Hughes 1995). The effects of IUD use on genetic diversity in a given herd should be comparable to those of other temporary fertility control methods; use should reduce the fraction of mares breeding at any one time, but does not necessarily preclude treated mares from breeding in the future, as they survive and regain fertility.

The exact mechanism by which IUDs prevent pregnancy is uncertain, but may be related to persistent, low-grade uterine inflammation (Daels and Hughes 1995, Gradil et al. 2021, Hoopes et al. 2021), Turner et al. (2015) suggested that the presence of an IUD in the uterus may, like a pregnancy, prevent the mare from coming back into estrus. However, some domestic mares did exhibit repeated estrus cycles during the time when they had IUDs (Killian et al. 2008, Gradil et al. 2019, Lyman et al. 2021, Hoopes et al. 2021). The main cause for an IUD to not be effective at contraception is its failure to stay in the uterus (Daels and Hughes 1995, NAS 2013). As a result, one of the major challenges to using IUDs to control fertility in mares on the range is preventing the IUD from being dislodged or otherwise ejected over the course of daily activities, which could include, at times, frequent breeding.

At this time, it is thought that any IUD inserted into a pregnant mare may cause the pregnancy to terminate, which may also cause the IUD to be expelled. For that reason, it is expected that IUDs would only be inserted in non-pregnant (open) mares. Wild mares receiving IUDs would be checked for pregnancy by a veterinarian prior to insertion of an IUD. This can be accomplished by transrectal palpation and/or ultrasound performed by a veterinarian. Pregnant mares would not receive an IUD. Only a veterinarian would apply IUDs in any BLM management action. The IUD is inserted into the uterus using a thin, tubular applicator similar to a shielded culture tube, and would be inserted in a manner similar to that routinely used to obtain uterine cultures in domestic mares. If a mare has a zygote or very small, early phase embryo, it is possible that it will fail to be detected in screening, and may develop further, but without causing the expulsion of the IUD. Wild mares with IUDs would be individually marked and identified, so that they can be monitored occasionally and examined, if necessary, in the future, consistent with other BLM management activities.

Using metallic or glass marbles as IUDs may prevent pregnancy in horses (Nie et al. 2003), but can pose health risks to domestic mares (Turner et al. 2015, Freeman and Lyle 2015). Marbles may break into shards (Turner et al. 2015), and uterine irritation that results from marble IUDs may cause chronic, intermittent colic (Freeman and Lyle 2015). Metallic IUDs may cause severe infection (Klabnik-Bradford et al. 2013).

In domestic ponies, Killian et al. (2008) explored the use of three different IUD configurations, including a silastic polymer O-ring with copper clamps, and the “380 Copper T” and “GyneFix” IUDs designed for women. The longest retention time for the three IUD models was seen in the “T” device, which stayed in the uterus of several mares for 3-5 years. Reported contraception rates for IUD-treated mares were 80%, 29%, 14%, and 0% in years 1-4, respectively. They surmised that pregnancy resulted after IUD fell out of the uterus. Killian et al. (2008) reported high levels of progesterone in non-pregnant, IUD-treated ponies.

Soft or flexible IUDs may cause relatively less discomfort than hard IUDs (Daels and Hughes 1995). Daels and Hughes (1995) tested the use of a flexible O-ring IUD, made of silastic, surgical-grade polymer, measuring 40 mm in diameter; in five of six breeding domestic mares tested, the IUD was reported to have stayed in the mare for at least 10 months. In mares with IUDs, Daels and Hughes (1995) reported some level of uterine irritation, but surmised that the level of irritation was not enough to interfere with a return to fertility after IUD removal.

More recently, several types of soft or flexible IUDs have been tested for use in breeding mares. When researchers attempted to replicate the O-ring study (Daels and Hughes 1995) in an USGS / Oklahoma State University (OSU) study with breeding domestic mares, using various configurations of silicone O-ring IUDs, the IUDs fell out at unacceptably high rates over time scales of less than 2 months (Baldrighi et al. 2017, Lyman et al. 2021). Subsequently, the USGS / OSU researchers tested a Y-shaped IUD to determine retention rates and assess effects on uterine health; retention rates were greater than 75% for an 18-month period, and mares returned to good uterine health and reproductive capacity after removal of the IUDs (Holyoak et al. 2021). These Y-shaped silicone IUDs are considered a pesticide device by the EPA, in that they work by physical means (EPA 2020). The University of Massachusetts has developed a magnetic IUD that has been effective at prolonging estrus and preventing pregnancy in domestic mares (Gradil et al. 2019, Joonè et al. 2021, Gradil et al. 2021, Hoopes et al. 2021). After insertion in the uterus, the three subunits of the device are held together by magnetic forces as a flexible triangle. A metal detector can be used to determine whether the device is still present in the mare. In an early trial, two sizes of those magnetic IUDs fell out of breeding domestic mares at high rates (Holyoak et al., unpublished results), but more recent trials have shown that the magnetic IUD was retained even in the presence of breeding with a fertile stallion (Hoopes et al. 2021). The magnetic IUD was used in two trials where mares were exposed to stallions, and in one where mares were artificially inseminated; in all cases, the IUDs were reported to stay in the mares without any pregnancy (Gradil 2019, Joonè et al. 2021, Gradil et al. 2021, Hoopes et al. 2021).

8.3 Sex Ratio Manipulation

Skewing the sex ratio of a herd so that there are more males than females is an established BLM management technique for reducing population growth rates. As part of a wild horse and burro gather process, the number of animals returned to the range may include more males, the number removed from the range may include more females, or both. By reducing the proportion of breeding females in a population (as a fraction of the total number of animals present), the technique leads to fewer foals being born, relative to the total herd size.

Sex ratio is typically adjusted in such a way that 60 percent of the horses are male. In the absence of other fertility control treatments, this 60:40 sex ratio can temporarily reduce population growth rates from approximately 20% to approximately 15% (Bartholow 2004). While such a decrease in growth rate may not appear to be large or long-lasting, the net result can be fewer foals being born, at least for a few years – this can extend the time between gathers, and reduce impacts on-range, and costs off-range. Any impacts of sex ratio manipulation are expected to be temporary because the sex ratio of wild horse and burro foals at birth is approximately equal between males and females (NAS 2013), and it is common for female foals to reproduce by their second year (NAS 2013). Thus, within a few years after a gather and selective removal that leads to more males than females, the sex ratio of reproducing wild horses and burros will be returning toward a 50:50 ratio.

Having a larger number of males than females is expected to lead to several demographic and behavioral changes as noted in the NAS report (2013), including the following. Having more fertile males than females should not alter the fecundity of fertile females. Wild mares may be distributed in a larger number of smaller harems. Competition and aggression between males may cause a decline in male body condition. Female foraging may be somewhat disrupted by elevated male-male aggression. With a greater number of males available to choose from, females may have opportunities to select more genetically fit sires. There would also be an increase in the genetic effective population size because more stallions would be breeding and existing females would be distributed among many more small harems. This last beneficial impact is one reason that skewing the sex ratio to favor males is listed in the BLM wild horse and burro handbook (BLM 2010) as a method to consider in herds where there may be concern about the loss of genetic diversity; having more males fosters a greater retention of genetic diversity.

Infanticide is a natural behavior that has been observed in wild equids (Feh and Munktuya 2008, Gray 2009), but there are no published accounts of infanticide rates increasing as a result of having a skewed sex ratio in wild horse or wild burro herds. Any comment that implies such an impact would be speculative.

The BLM wild horse and burro management handbook (BLM 2010) discusses this method. The handbook acknowledges that there may be some behavioral impacts of having more males than females. The handbook includes guidelines for when the method should be applied, specifying that this method should be considered where the low end of the AML is 150 animals or greater, and with the result that males comprise 60-70 percent of the herd. Having more than 70 percent males may result in unacceptable impacts in terms of elevated male-male aggression. In NEPA analyses, BLM has chosen to follow these guidelines in some cases, for example:

- In the 2015 Cold Springs HMA Population Management Plan EA (DOI-BLM-V040-2015-022), the low end of AML was 75. Under the preferred alternative, 37 mares and 38 stallions would remain on the HMA. This is well below the 150 head threshold noted above.
- In the 2017 Hog Creek HMA Population Management Plan EA (DOI-BLM-ORWA-V000-2017-0026-EA), BLM clearly identified that maintaining a 50:50 sex ratio was appropriate because the herd size at the low end of AML was only 30 animals.

It is relatively straightforward to speed the return of skewed sex ratios back to a 50:50 ratio. The BLM wild horse and burro handbook (BLM 2010) specifies that, if post-treatment monitoring reveals negative impacts to breeding harems due to sex ratio manipulation, then mitigation measures could include removing males, not introducing additional males, or releasing a larger proportion of females during the next gather.

9.0 Fertility Control Treatment (SOPs)

SOPs common to all vaccine types:

Identification
Animals intended for treatment must be clearly, individually identifiable to allow for positive identification during subsequent management activities. For captured animals, marking for identification may be accomplished by marking each individual with a freeze mark on the hip and/ or neck and a microchip in the nuchal ligament. In some cases, identification may be accomplished Such animals may be photographed using a telephoto lens and high quality digital camera as a record of treated individuals.

Safety
Safety for both humans and animals is the primary consideration in all elements of fertility control vaccine use. Administration of any vaccine must follow all safety guidance and label guidelines on applicable EPA labeling.

Injection Site
For hand-injection, delivery of the vaccine should be by intramuscular injection, while the animal is standing still, into the left or right side, above the imaginary line that connects the point of the hip (hook bone) and the point of the buttocks (pin bone): this is the hip / upper gluteal area. For dart-based injection, delivery of the vaccine should be by intramuscular injection, while the animal is standing still, into the left or right thigh areas (lower gluteal / biceps femoralis).

Monitoring and Tracking of Treatments

1. Estimation of population size and growth rates (in most cases, using aerial surveys) should be conducted periodically after treatments.
2. Population growth rates of some herds selected for intensive monitoring may be estimated every year post-treatment using aerial surveys. If, during routine HMA field monitoring (on-the-ground), data describing adult to foal ratios can be collected, these data should also be shared with HQ-261.
3. Field applicators should record all pertinent data relating to identification of treated animals (including photographs if animals are not freeze-marked) and date of treatment, lot number(s) of the vaccine, quantity of vaccine issued, the quantity used, the date of vaccination, disposition of any unused vaccine, the date disposed, the number of treated mares by HMA, field office, and State along with the microchip numbers and freeze-mark(s) applied by HMA and date. A summary narrative and data sheets will be forwarded to HQ-261 annually (Reno, Nevada). A copy of the form and data sheets and any photos taken should be maintained at the field office.

HQ-261 will maintain records sent from field offices, on the quantity of PZP issued, the quantity used, disposition of any unused PZP, the number of treated mares by HMA, field office, and State along with the freeze-mark(s) applied by HMA and date.

PZP Vaccine SOPs

1. PZP vaccine would be administered by trained BLM personnel.

2. The fertility control drug is administered with two separate injections: (1) a liquid dose of PZP is administered using an 18-gauge needle primarily by hand injection; (2) the pellets are preloaded into a 14-gauge needle. These are loaded on the end of a trocar (dry syringe with a metal rod) which is loaded into the jab-stick which then pushes the pellets into the breeding mares being returned to the range. The pellets and liquid are designed to release the PZP over time similar to a time-release cold capsule.

3. Delivery of the vaccine would be as an intramuscular injection while the mares are restrained in a working chute. Half a cubic centimeter (cc) of the PZP vaccine would be emulsified with half a cc of adjuvant (a compound that stimulates antibody production) and loaded into the delivery system. The pellets would be loaded into the jab-stick for the second injection. With each injection, the liquid and pellets would be propelled into the left hindquarters of the mare, just below the imaginary line that connects the point of the hip and the point of the buttocks.

4. All treated mares would be freezemarked on the hip and / or chipped to enable researchers to positively identify the animals during the research project as part of the data collection phase.

5. At a minimum, monitoring of reproductive rates using helicopter flyovers will be conducted in years two through four by checking for the presence or absence of foals. The flight scheduled for year four will also assist in determining the percentage of mares that have returned to fertility. In addition, field monitoring will be routinely conducted as part of other regular ground-based monitoring activities.

6. A field data sheet will be used by the field applicators to record all the pertinent data relating to identification of the mare including a photograph when possible, date of treatment, type of treatment (1 or 2 year vaccine, adjuvant used) and HMA. The original form with the data sheets will be forwarded to the Authorized Officer at the National Program Office (NPO) in Reno, Nevada. A copy of the form and data sheets and any photos taken will be maintained at the district office.

7. A tracking system will be maintained by NPO detailing the quantity of PZP issued, the quantity used, and disposition of any unused PZP, the number of treated mares by HMA, district office, and state along with the freeze-mark and / or chip applied by HMA.

8. The field office will assure that treated mares do not enter the adoption market for 3 years following treatment. In the rare instance, due to unforeseen circumstances, that treated mare(s) are removed from an HMA before 3 years have lapsed, they will be maintained in either a BLM facility or BLM-contracted Long-Term Pastures (LTPs) until expiration of the 3-year holding period. In the event it is necessary to remove treated mares, their removal and disposition will be coordinated through NPO. After expiration of the 3-year holding period, the animal may be placed in the adoption program or sent to long-term pastures.

PZP Remote Darting SOPs

1. PZP vaccine would be administered through darting by trained BLM personnel or collaborating partners only. For any darting operation, the designated personnel must have successfully completed a nationally recognized wildlife darting course and who have documented and successful experience darting wildlife under field conditions.

2. All mares targeted for treatment will be clearly identifiable through photographs to enable darters and HMA managers to positively identify the animals during the project and at the time of removal during subsequent gathers.

3. Mares that have never been treated would receive 0.5 cc of PZP vaccine emulsified with 0.5 cc of Freund's Modified Adjuvant (FMA) and loaded into darts at the time a decision has been made to dart a specific mare. Mares identified for re-treatment receive 0.5 cc of the PZP vaccine emulsified with 0.5 cc of Freund's Incomplete Adjuvant (FIA).

4. The liquid dose of PZP vaccine is administered using 1.0 cc Pneu-Darts with 1.25" or 1.5" barbless needles fired from either Dan Inject®, Pneu-Dart® X-Caliber or Palmer® Cap-Chur rifle.

5. Only designated darters would mix the vaccine/adjuvant and prepare the emulsion. Vaccine-adjuvant emulsion would be loaded into darts at the darting site and delivered by means of an appropriate CO_2 powered or cartridge darting delivery system.

6. Delivery of the vaccine would be by intramuscular injection into the left or right hip/gluteal muscles while the mare is standing still.

7. Safety for both humans and the horse is the foremost consideration in deciding to dart a mare. Safe darting distances would depend on the skill and ability of the darter, and the particular model of dart gun being utilized. No attempt would be taken when other persons are within a 30-m radius of the target animal.

8. No attempts would be taken in high wind or when the horse is standing at an angle where the dart could miss the hip/gluteal region and hit the rib cage. The ideal is when the dart would strike the skin of the horse at a perfect 90° angle.

9. If a loaded dart is not used within two hours of the time of loading, the contents would be transferred to a new dart before attempting another horse. If the dart is not used before the end of the day, it would be stored under refrigeration and the contents transferred to another dart the next day. Refrigerated darts would not be used in the field.

10. No more than two people should be present at the time of a darting. The second person is responsible for locating fired darts. The second person should also be responsible for identifying the horse and keeping onlookers at a safe distance.

11. To the extent possible, all darting would be carried out in a discrete manner. However, if darting is to be done within view of non-participants or members of the public, an explanation of the nature of the project would be carried out either immediately before or after the darting.

12. Attempts will be made to recover all darts. To the extent possible, all darts which are discharged and drop from the horse at the darting site would be recovered before another darting occurs. In exceptional situations, the site of a lost dart may be noted and marked, and recovery efforts made at a later time. All discharged darts would be examined after recovery in order to determine if the charge fired and the plunger fully expelled the vaccine. Personnel conducting darting operations should be equipped with a two-way radio or cell phone to provide a communications link with the Project Veterinarian for advice and/or assistance. In the event of a veterinary emergency, darting personnel would immediately contact

the Project Veterinarian, providing all available information concerning the nature and location of the incident.

13. In the event that a dart strikes a bone or imbeds in soft tissue and does not dislodge, the darter would follow the affected horse until the dart falls out or the horse can no longer be found. The darter would be responsible for daily observation of the horse until the situation is resolved.

GonaCon SOPs

GonaCon-Equine vaccine (USDA Pocatello Storage Depot, Pocatello, ID; Spay First!, Inc., Oklahoma City, OK) is distributed as preloaded doses (2 mL) in labeled syringes.

Delivering GonaCon by Hand-Injection of GonaCon

1. GonaCon-Equine vaccine is administered by hand-injection to mares that are appropriately immobilized or restrained. Important: label instructions must be followed for this product. Females identified for treatment application are hand-injected with an intramuscular injection of Gona-Equine vaccine (2 ml) in the lower gluteal musculature using a hand-held, luer-lock syringe (18-gauge, 3.8 cm needle). The syringe is made of transparent plastic with the barrel showing graduated marks indicating the volume of the vaccine in the syringe. This facilitates the visual assessment of the quantity of vaccine injected into the animal without the need to weigh the syringes. Pre-loaded syringes should be kept refrigerated overnight and then set out the morning of application at room temperature. They should not be allowed to get too warm or cold during the day.

2. The vaccine is distributed as preloaded doses (2 mL) in labeled syringes. Upon receipt, the vaccine should be kept refrigerated (4° C) until use. Do not freeze. The vaccine has a 6-month shelf-life from the time of production and the expiration date will be noted on each syringe that is provided.

3. Although infrequent, hand-injections to immobilized or restrained horses can result in partial delivery of the vaccine due to inexperienced personnel and/or unexpected movement of the horse. As a precaution, order extra doses of the vaccine. For hand-injection application, assume a 10% failure rate and increase the original quantity accordingly.

4. Examine each syringe before and after injection and visually determine approximately how much vaccine was injected. A full dose is considered 90% (1.8 ml) or greater of the original 2 ml dose. Ensure a full dose is administered.

5. It is recommended that all treated mares be photographed to facilitate identification by individual markings, RFID chip, and/or freeze-marked on the hip or neck to positively identify the animals as a GonaCon-Equine vaccinated mare during field observations or subsequent gathers.

Preparation of Darts for GonaCon Remote Delivery:

General practice guidelines for darting operations, as noted above for dart-delivery of ZonaStat-H, should be followed for dart-delivery of GonaCon-Equine.

1. The vaccine is distributed as preloaded doses (2 mL) in labeled syringes. Upon receipt, the vaccine should be kept refrigerated (4° C) until use. Do not freeze. The vaccine has a 6-month shelf-life from the time of production and the expiration date will be noted on each syringe that is provided. Important: label instructions must be followed for this product.

2. Although infrequent, dart injections can result in partial injections of the vaccine, and shots are missed. As a precaution, it is recommended that extra doses of the vaccine be ordered to accommodate failed delivery (~15 %). To determine the amount of vaccine delivered, the dart must be weighed before loading, and before and after delivery in the field.

3. For best results, darts with a gel barb should be used. (i.e. 2 cc Pneu-Dart brand darts configured with Slow-inject technology, 3.81 cm long 14 ga.tri-port needles, and gel collars positioned 1.27 cm ahead of the ferrule).

4. Wearing latex gloves, darts are numbered and filled with vaccine by attaching a loading needle (7.62 cm; provided by dart manufacturer) to the syringe containing vaccine and placing the needle into the cannula of the dart to the fullest depth possible. Slowly depress the syringe plunger and begin filling the dart. Periodically, tap the dart on a hard surface to dislodge air bubbles trapped within the vaccine. Due to the viscous nature of the fluid, air entrapment typically results in a maximum of approximately 1.8 ml of vaccine being loaded in the dart. The dart is filled to max once a small amount of the vaccine can be seen at the tri-ports.

5. Important! Do not load and refrigerate darts the night before application. When exposed to moisture and condensation, the edges of gel barbs soften, begin to dissolve, and will not hold the dart in the muscle tissue long enough for full injection of the vaccine. The dart needs to remain in the muscle tissue for a minimum of 1 minute to achieve dependable full injection. Sharp gel barbs are critical.

6. Darts (configured specifically as described above) can be loaded in the field and stored in a cooler prior to application. Darts loaded, but not used can be maintained in a cooler at about 4° C and used the next day, but do not store in a refrigerator or any other container likely to cause condensation.

Administering the GonaCon Vaccine Remotely (via Darting):

1- For initial and booster treatments, mares would ideally receive 2.0 ml of GonaCon-Equine. However, experience has demonstrated that only 1.8 ml of vaccine can typically be loaded into 2 cc darts, and this dose has proven successful. Calculations below reflect a 1.8 ml dose.

2- With each injection, the vaccine should be injected into the left or right hind quarters of the mare, above the imaginary line that connects the point of the hip (hook bone) and the point of the buttocks (pin bone).

3- Darts should be weighed to the nearest hundredth gram by electronic scale when empty, when loaded with vaccine, and after discharge, to ensure that 90% (1.62 ml) of the vaccine has been injected. Animals receiving <50% should be darted with another full dose; those receiving >50% but <90% should receive a half dose (1 ml). All darts should be weighed to verify a combination of ≥1.62 ml has been administered. Therefore, every effort should be made to recover darts after they have fallen from animals.

4- A booster vaccine may be administered after the first injection to improve efficacy of the product over subsequent years.

5- Free ranging animals may be photographed using a telephoto lens and high quality digital receiver as a record of treated individuals, and the injection site can be recorded on data sheets to facilitate

identification by animal markings and potential injection scars.

SOPs for Insertion of Y-shaped Silicone IUD for Feral Horses

Background: Mares must be open. A veterinarian must determine pregnancy status via palpation or ultrasound. Ultrasound should be used as necessary to confirm open status of mares down to at least 14 days for those that have recently been with stallions. For mares segregated from stallions, this determination may be made at an earlier time when mares are identified as candidates for treatment, or immediately prior to IUD insertion. Pregnant mares should not receive an IUD.

Preparation: IUDs must be clean and sterile. Sterilize IUDs with a low-temperature sterilization system, such as Sterrad.

The Introducer is two PVC pipes. The exterior pipe is a 29" length of ½" diameter pipe, sanded smooth at one end, then heat-treated to smooth its curvature further (Fig. 1). The IUD will be placed into this smoothed end of the exterior pipe. The interior pipe is a 29 ½" long, ¼" riser tube (of the kind used to connect water lines to sinks), with one end slightly flared out to fit more snugly inside the exterior pipe (Fig. 1), and a plastic stopper attached to the other end (Fig. 2).

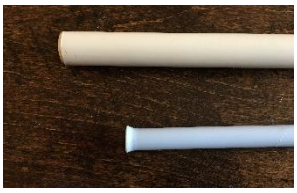


Figure 1. Interior and exterior pipes (unassembled), showing the ends that go into the mare

Figure 2. Interior pipe shown within exterior pipe. After the introducer is 4" beyond the os, the stopper is pushed forward (outside the mare), causing the IUD to be pushed out from the exterior pipe.

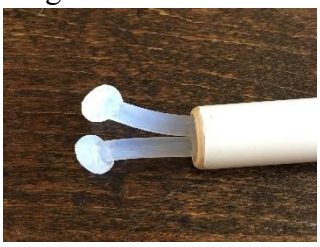
Introducers should be sterilized in Benz-all cold steriliant, or similar. Do not use iodine-based sterilant solution. A suitable container for sterilant can be a large diameter (i.e., 2") PVC pipe with one end sealed and one end removable.

Prepare the IUD: Lubricate with sterile veterinary lube, and insert into the introducer. The central stem of the IUD goes in first (Fig. 3).

Figure 3. Insert the stem end of the IUD into the exterior pipe.

Fold the two 'legs' of the IUD, and push the IUD further into the introducer, until just the bulbous ends are showing (Fig. 4).

Figure 4. Insert the IUD until just the tips of the 'legs' are showing.



Restraint and Medication: The mare should be restrained in a padded squeeze chute to provide access to the rear end of the animal, but with a solid lower back door, or thick wood panel, for veterinarian safety.

Only a veterinarian shall oversee this procedure and insert IUDs. Some veterinary practitioners may choose to provide sedation. If so, when the mare's head starts to droop, it may be advisable to tie the tail up to prevent risk of the animal sitting down on the veterinarian's arm (i.e., double half hitch, then tie tail to the bar above the animal). Some veterinary practitioners may choose to provide a dose of long-acting progesterone to aid in IUD retention. Example dosage: 5mL of BioRelease LA Progesterone 300 mg/mL (BET labs, Lexington KY), *or* long-acting Altrenogest). No other intrauterine treatments of any kind should be administered at the time of IUD insertion.

Insertion Procedure:

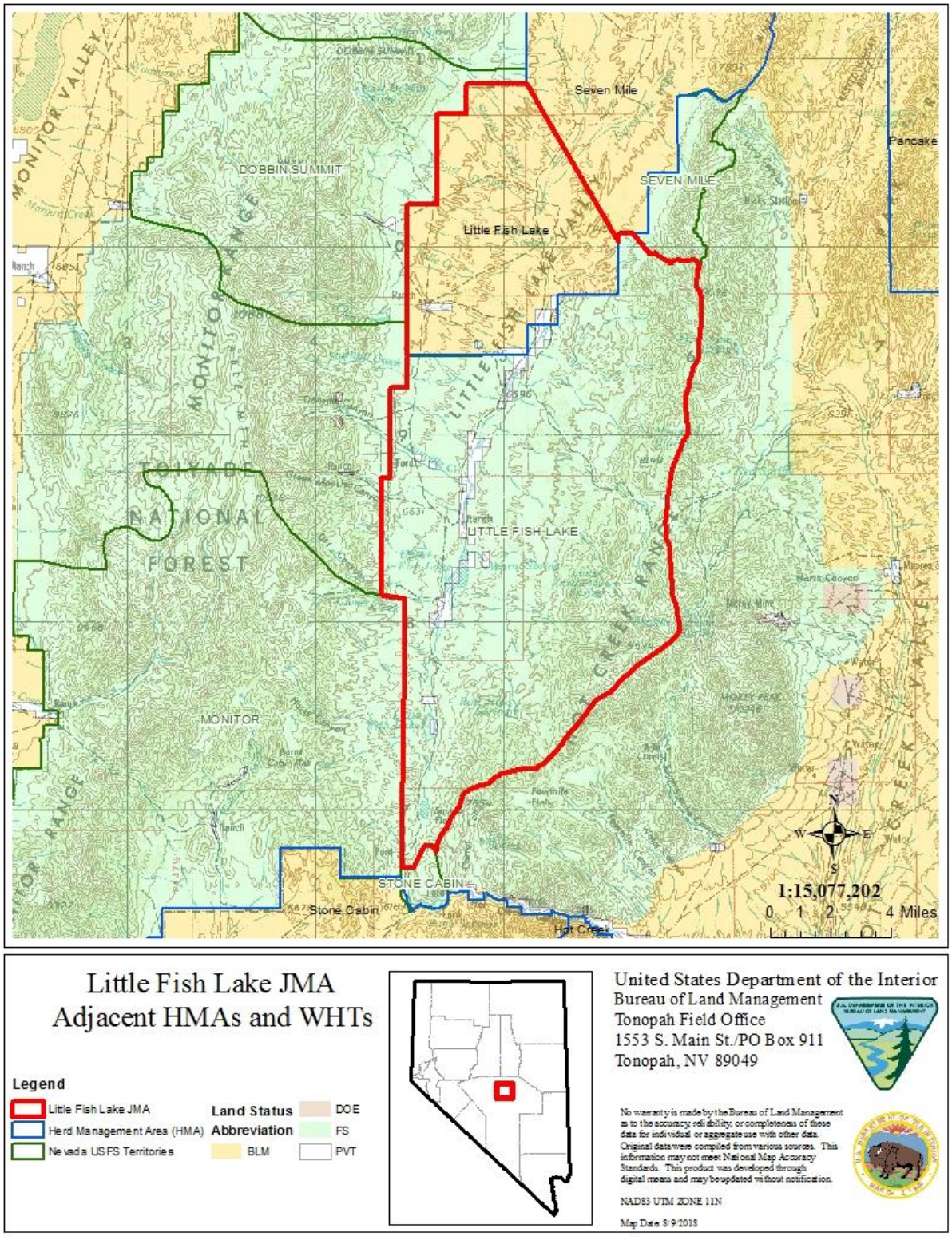
- Prep clean the perineal area.
- Lubricate the veterinarian's sleeved arm and the Introducer+IUD.
- Carry the introducer (IUD-end-first) into the vagina.
- Dilate the cervix and gently move the tip of the introducer past the cervix.
- Advance the end of the 1/2" PVC pipe about 4 inches past the internal os of the cervix.
- Hold the exterior pipe in place, but push the stopper of the interior pipe forward, causing the IUD to be pushed out of the exterior pipe, into the uterus.
- Placing a finger into the cervical lumen just as the introducer tube is removed from the external os allows the veterinarian to know that the IUD is left in the uterus, and not dragged back into or past the cervix.
- Remove the introducer from the animal, untie the tail.

Mares that have received an IUD should be observed closely for signs of discharge or discomfort for 24 hours following insertion after which they may be released back to the range.

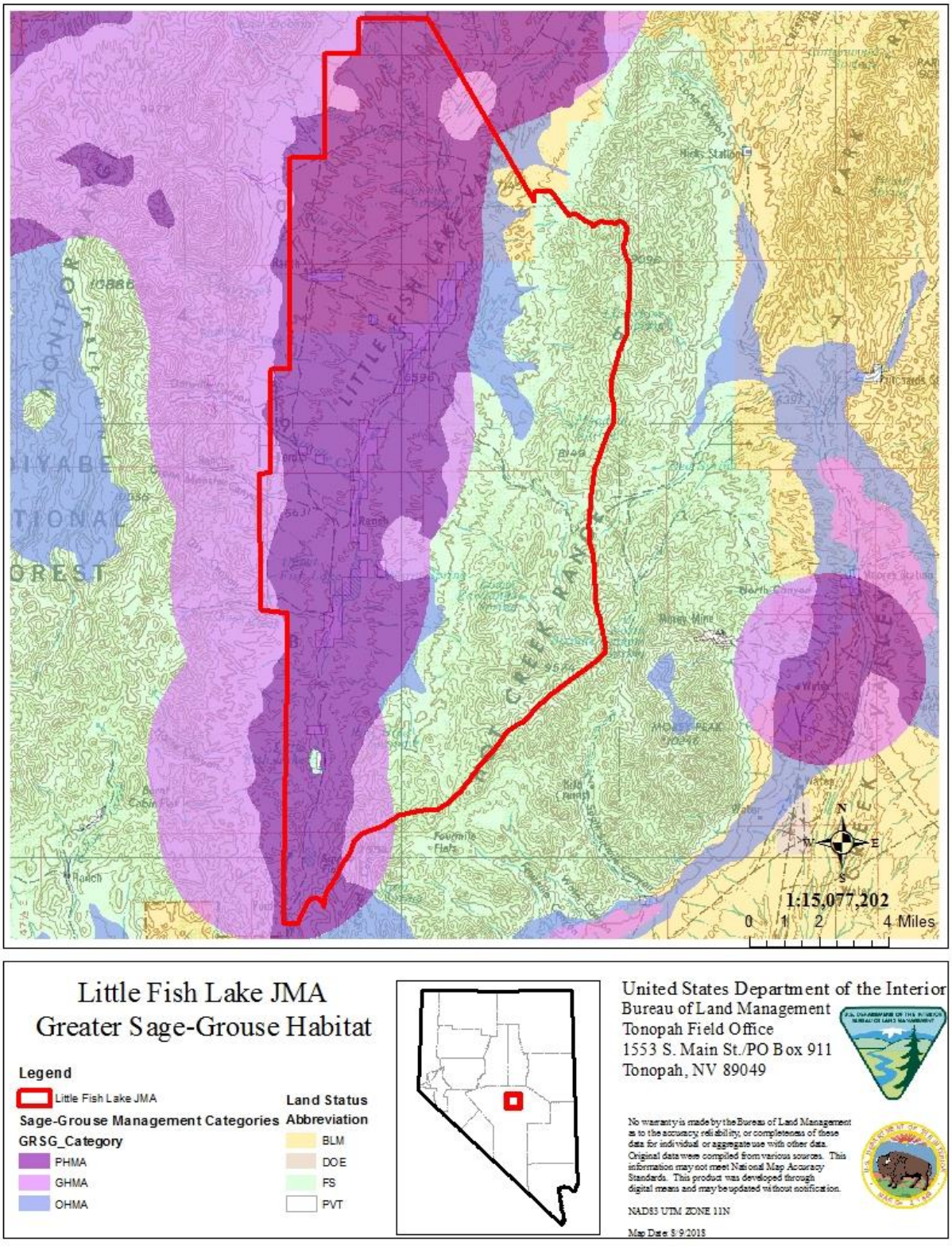
10.0 Literature Cited

Literature cited for both the EA document and the Supplemental Information Report is located in Section 8 of the EA.

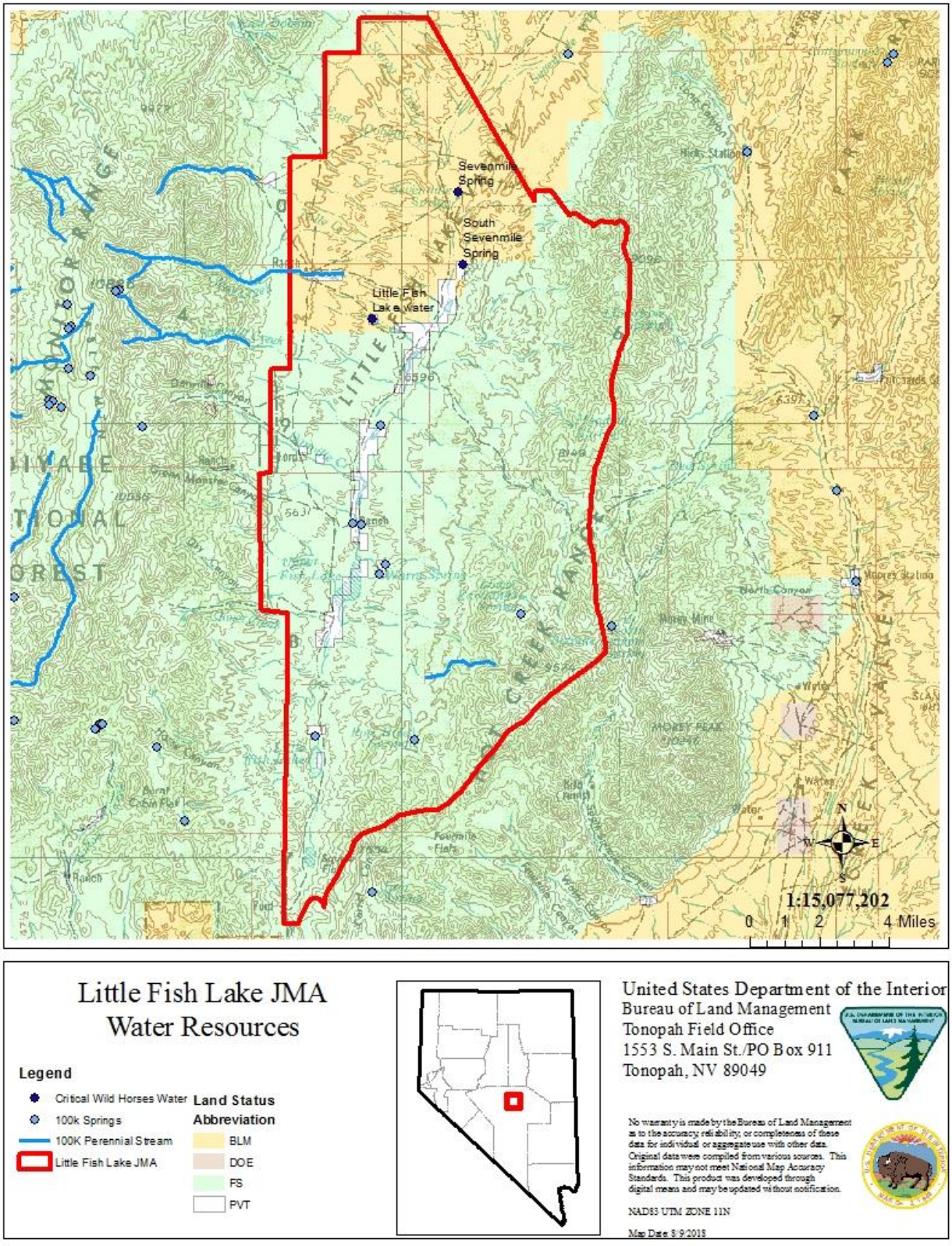
11.0 Maps



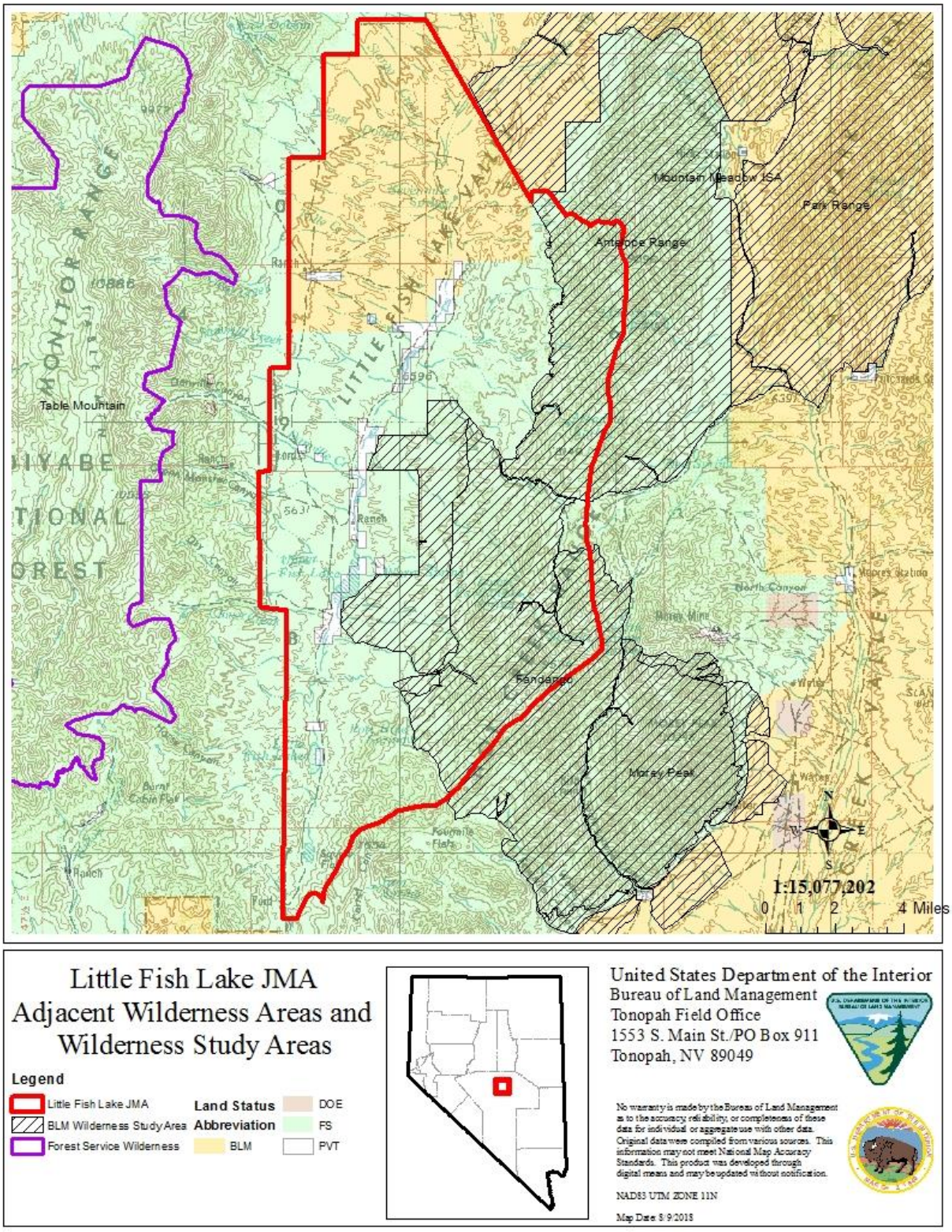
Map 4. Little Fish Lake JMA and adjacent HMAs and WHTs



Map 5. Greater Sage-Grouse Habitat within and around the Little Fish Lake JMA



Map 6. Little Fish Lake Water Resources



Map 7. Little Fish Lake JMA adjacent wilderness areas and wilderness study areas.